

BI

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 September 2002 (26.09.2002)

PCT

(10) International Publication Number
WO 02/074992 A2

- (51) International Patent Classification⁷: **C12Q 1/68** (IS). JONSDOTTIR, Sif [IS/IS]; Vesturgata 73, IS-101 Reykjavik (IS). REYNISDOTTIR, Sigridur, Th. [IS/IS]; Storgærði 8, IS-108 Reykjavik (IS).
- (21) International Application Number: PCT/IB02/00565
- (22) International Filing Date: 25 February 2002 (25.02.2002) (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
09/811,352 19 March 2001 (19.03.2001) US
10/067,514 4 February 2002 (04.02.2002) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:
US 09/811,352 (CON)
Filed on 19 March 2001 (19.03.2001)
US Not furnished (CON)
Filed on 4 February 2002 (04.02.2002)
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- Published:**
— without international search report and to be republished upon receipt of that report
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/074992 A2

(54) Title: HUMAN STROKE GENE

(57) Abstract: A role of the human PDE4D gene in stroke is disclosed. Methods for diagnosis, prediction of clinical course and treatment for stroke using polymorphisms in the PDE4D gene are also disclosed.

HUMAN STROKE GENE

RELATED APPLICATION

This is a continuation of U.S. Application _____ (2345.2010-003), which was filed on February 4, 2002, which is a continuation-in-part of U.S.

- 5 Application No. 09/811,352, filed March 19, 2001. The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Stroke is a major health problem in western societies. It is the leading cause of disability, the second leading cause of dementia and the third most common cause of death (Bonita, R., *Lancet* 339:342 (1992)). As it is more common in the elderly, the public health impact of stroke will increase in the next decades with growing life expectancy. Almost 1 out of 4 men and nearly 1 out of 5 women aged 45 years will have a stroke if they live to their 85th year (Bonita, R., *Lancet* 339:342 (1992)). Strategies to diminish the impact of stroke includes prevention and treatment with thrombolytics and possibly neuroprotective agents. The success of preventive measures will depend on the identification of risk factors and means to modulate their risk.

The clinical phenotype of stroke is complex but can be broadly divided into ischemic and hemorrhagic stroke. The majority of strokes (80 to 90%) are ischemic, caused by obstruction of blood flow through extra- or intracranial vessels (Mohr, J.P., *et al.*, *Neurology*, 28:754-762 (1978); Caplan, L.R., *In Stroke, A Clinical Approach* (Butterworth-Heinemann, Stoneham, MA, ed 3, 1993)). The remainder are hemorrhagic strokes (10-20%), resulting from ruptures of intracranial vessels. Ischemic stroke can be further subdivided into large vessel occlusive disease, small vessel occlusive disease, and cardiogenic stroke. Transient ischemic attack (TIA), although not defined as a stroke because the signs and symptoms (which are the same as for stroke) last for a short period of time (less than 24 hours, usually 5 to 20

minutes), indicates a serious underlying risk that a stroke may follow, and it is believed that the same pathophysiologic mechanisms are responsible for TIA and ischemic stroke (Caplan, L.R., *In Stroke, A Clinical Approach* (Butterworth-Heinemann, Stoneham, MA, ed 3, 1993)).

- 5 The predominant risk factor for all types of stroke is hypertension (Thompson, D.W. and A.J. Furlan, *Neurosurg. Clin. N. Am.*, 8:265-269 (1997); Agnarsson, U., *et al.*, *Ann. Intern. Med.*, 130:987 (1999)). Hypertension is in itself a complex disease as are the other known secondary risk factors, diabetes and hyperlipidemia. In addition, there are environmental risk factors such as smoking.
- 10 Stroke is therefore considered to be a highly complex disease consisting of a group of heterogeneous disorders with multiple risk factors, genetic and environmental.

- The identification of genetic determinants of common diseases such as stroke, which may result from an interplay among multiple genes and between genes and environmental risk factors, has proven to be a difficult task. Studies of the
- 15 genetic contribution to stroke have mainly focused on rare Mendelian diseases where stroke is a part of the phenotype or on finding association with possible candidate genes such as genes contributing to hypertension or lipid metabolism. Several genes have been identified that play roles in the pathogenesis of rare stroke syndromes such as the *Notch3* gene in CADASIL (cerebral autosomal dominant arteriopathy
- 20 with subcortical infarctions and leukoencephalopathy) (Tournier-Lasserre, E., *et al.*, *Nat. Genet.*, 3:256-259 (1993); Joutel, A., *et al.*, *Nature*, 383:707 (1996)), *Cystatin C* in the Icelandic type of hereditary cerebral hemorrhage with amyloidosis (Palsdottir, A., *et al.*, *Lancet*, 2:603-604 (1998)), *APP* in the Dutch type of hereditary cerebral hemorrhage (Levy, E., *et al.*, *Science*, 248:1124 (1990)), and the
- 25 *KRIT1* gene in patients with hereditary cavernous angioma (Gunel, M., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 92:6620-6624 (1995); Laberge-le Couteux, S., *et al.*, *Nat. Genet.* 23:189 (1999); Sahoo, T., *et al.*, *Hum. Mol. Genet.* 8:2325 (1999)).

- In addition to family history information for stroke, it is desirable to develop diagnostic methods for the early diagnosis of the disease or predisposition for the
- 30 development of stroke. Better means for predicting and identifying stroke should lead to better prophylactic and treatment regimens.

SUMMARY OF THE INVENTION

As described herein, it has been discovered that the gene that encodes phosphodiesterase 4D (hereinafter referred to as "PDE4D") has been correlated through human linkage studies to stroke, particularly ischemic strokes and transient
5 ischemic attacks. Five new exons, here referred to as 4D7-1, 4D7-2, 4D7-3, 4D6 and 4D8 have been identified. Three novel splice variants have also been identified (see Fig. 4).

The present invention relates to isolated nucleic acid molecules comprising the PDE4D gene. In one embodiment, the isolated nucleic acid molecule comprises
10 a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10, and the complement thereof. The invention further relates to a nucleic acid molecule which hybridizes under high stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally
15 comprise at least one polymorphism as shown in Tables 9 and 10, and the complement thereof. The invention additionally relates to isolated nucleic acid molecules (e.g., cDNA molecules) encoding a PDE4D polypeptide (e.g., encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14 or another splicing variant of PDE4D polypeptide which includes a polymorphic site and/or novel exon selected from the
20 group consisting of 4D6, 4D7-1, 4D7-2, 4D7-3 and 4D8).

The invention further provides a method for assaying a sample for the presence of a nucleic acid molecule comprising all or a portion of PDE4D in a sample, comprising contacting said sample with a second nucleic acid molecule comprising a nucleotide sequence encoding a PDE4D polypeptide (e.g., SEQ ID
25 NO: 1 or the complement of SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10; a nucleotide sequence encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10, or another splicing variant of PDE4D polypeptide which includes a polymorphic site and/or exon selected from
30 the group consisting of 4D6, 4D7-1, 4D7-2, 4D7-3 and 4D8), or a fragment or derivative thereof, under conditions appropriate for selective hybridization. The

invention additionally provides a method for assaying a sample for the level of expression of a PDE4D polypeptide, or fragment or derivative thereof, comprising detecting (directly or indirectly) the level of expression of the PDE4D polypeptide, fragment or derivative thereof.

5 The invention also relates to a vector comprising an isolated nucleic acid molecule of the invention operatively linked to a regulatory sequence, as well as to a recombinant host cell comprising the vector. The invention also provides a method for preparing a polypeptide encoded by an isolated nucleic acid molecule described herein (an PDE4D polypeptide), comprising culturing a recombinant host cell of the
10 invention under conditions suitable for expression of said nucleic acid molecule.

 The invention further provides an isolated polypeptide encoded by isolated nucleic acid molecules of the invention (e.g., PDE4D polypeptide), as well as fragments or derivatives thereof. In a particular embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:
15 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14 and containing at least one polymorphism described herein, particularly a polymorphism in all or a portion of exon 4D1, such as a SNP at 1,591,306, or one or a combination of SNPs in Table 5B. In another embodiment, the polypeptide is another splicing variant of an
20 PDE4D polypeptide, particularly a splicing variant containing all or a portion of exon selected from the group consisting of, 4D7-1, 4D7-2, 4D7-3 and 4D8. The invention also relates to an isolated polypeptide comprising an amino acid sequence which is greater than about 90 percent identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID
25 NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14 and containing at least one polymorphism described herein, particularly a polymorphism in all or a portion of exon 4D1, such as a SNP at 1,591,306, or one or a combination of SNPs in Table 5B; preferably about 95 percent identical.

 The invention also relates to an antibody, or an antigen-binding fragment
30 thereof, which selectively binds to a polypeptide of the invention, as well as to a method for assaying the presence of a polypeptide encoded by an isolated nucleic

acid molecule of the invention in a sample, comprising contacting said sample with an antibody which specifically binds to the encoded polypeptide.

The invention further relates to methods of diagnosing a predisposition to stroke. The methods of diagnosing a predisposition to stroke in an individual
5 include detecting the presence of a mutation in PDE4D, as well as detecting alterations in expression of an PDE4D polypeptide, such as the presence of different splicing variants of PDE4D polypeptides. The alterations in expression can be quantitative, qualitative, or both quantitative and qualitative. The methods of the invention allow the accurate diagnosis of stroke at or before disease onset, thus
10 reducing or minimizing the debilitating effects of stroke.

The invention additionally relates to an assay for identifying agents which alter (e.g., enhance or inhibit) the activity or expression of one or more PDE4D polypeptides. For example, a cell, cellular fraction, or solution containing an PDE4D polypeptide or a fragment or derivative thereof, can be contacted with an
15 agent to be tested, and the level of PDE4D polypeptide expression or activity can be assessed. The activity or expression of more than one PDE4D polypeptides can be assessed concurrently (e.g., the cell, cellular fraction, or solution can contain more than one type of PDE4D polypeptide, such as different splicing variants, and the levels of the different polypeptides or splicing variants can be assessed).

20 In another embodiment, the invention relates to assays to identify polypeptides which interact with one or more PDE4D polypeptides. In a yeast two-hybrid system, for example, a first vector is used which includes a nucleic acid encoding a DNA binding domain and also an PDE4D polypeptide, splicing variant, or fragment or derivative thereof, and a second vector is used which includes a
25 nucleic acid encoding a transcription activation domain and also a nucleic acid encoding a polypeptide which potentially may interact with the PDE4D polypeptide, splicing variant, or fragment or derivative thereof (e.g., a PDE4D polypeptide binding agent or receptor). Incubation of yeast containing both the first vector and the second vector under appropriate conditions allows identification of polypeptides
30 which interact with the PDE4D polypeptide or fragment or derivative thereof, and thus can be agents which alter the activity of expression of an PDE4D polypeptide.

Agents that enhance or inhibit PDE4D polypeptide expression or activity are also included in the current invention, as are methods of altering (enhancing or inhibiting) PDE4D polypeptide expression or activity by contacting a cell containing PDE4D and/or polypeptide, or by contacting the PDE4D polypeptide, with an agent
5 that enhances or inhibits expression or activity of PDE4D or polypeptide.

Additionally, the invention pertains to pharmaceutical compositions comprising the nucleic acids of the invention, the polypeptides of the invention, and/or the agents that alter activity of PDE4D polypeptide. The invention further pertains to methods of treating stroke, by administering PDE4D therapeutic agents,
10 such as nucleic acids of the invention, polypeptides of the invention, the agents that alter activity of PDE4D polypeptide, or compositions comprising the nucleic acids, polypeptides, and/or the agents that alter activity of PDE4D polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention
15 will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings.

Figs. 1A and 1B show two family pedigrees each affected by several of the stroke subtypes, including hemorrhagic stroke.

Figs. 2A, 2B and 2C show the genetic, combined and physical maps for
20 locating the PDE4D gene using 30 polymorphic markers. For the combined map, all markers have been assigned in the genetic and physical map unless otherwise indicated. (* indicates markers only assigned in physical map; ** indicates markers only assigned in genetic map).

Fig. 3 shows the genetic map of the stroke locus with exons and polymorphic
25 markers indicated. Markers identified by asterisks show association. The area defined by one drop in lod is approximately 4.6 Mb (approximately 5-6 cM).

Fig. 4 shows schematic representations of PDE4D splice variants. Splice variants 4D6, 4D7 and 4D8 are novel, as well as exons 4D6, 4D7-1, 4D7-2, 4D7-3 and 4D8. Splice variants 4DN1, 4DN2 and 4DN3 (Miro, *et al.*, *Biochem. Biophys.*

Res. Comm., 274:415-421 (2000)), and 4D1, 4D2, 4D3, 4D4 and 4D5 (Bolger *et al.*, *Biochem. J.*, Pt2:539-548 (1997) are known.

Fig. 5 is a schematic representation of the genetic map showing microsatellites and SNP haplotypes within the stroke gene.

5 Figs. 6.1 to 6.351 show the genomic sequence of the human PDE4D gene.

Figs. 7.1 to 7.10 show the amino acid sequences for the isoforms of the PDE4D gene. SEQ ID NO: 2 is D4; SEQ ID NO: 3 is N2; SEQ ID NO: 4 is D5; SEQ ID NO: 5 is N3; SEQ ID NO: 6 is D3; SEQ ID NO: 7 is N1; SEQ ID NO: 8 is D6; SEQ ID NO: 9 is D1; and SEQ ID NO: 10 is D2.

10 Figs. 8A and 8B list all publically available PDE4D2 mRNA's and novel eDNA segments identified by deCODE genetics.

DETAILED DESCRIPTION OF THE INVENTION

Extensive genealogical information for a population with population-based lists of patients has been combined with powerful genome sharing methods to map
 15 the first major locus in common stroke. A genome wide scan on patients, related within 6 meiotic events, diagnosed with stroke (ischemic and TIA) and their unaffected relatives has been completed. Locus *STRK1* on chromosome 5q12 has been identified through linkage studies to be associated with stroke. This locus does not correspond to known susceptibility loci for stroke or its risk factors (such as
 20 diabetes, hyperlipidemia and hypertension), and represents the first mapping of a gene for common stroke. Until now there have been no known linkage studies of stroke in humans showing any connection to this region of the chromosome. Based on the linkage studies conducted, Applicants have discovered a direct relationship between the PDE4D gene and stroke. Although the PDE4D gene (i.e., cDNA but
 25 not the genomic sequence) from normal individuals is known, there have been no studies directly investigating PDE4D and stroke. Moreover, there have been no variant forms reported that have been associated with stroke. The full sequence of the PDE4D gene and splice variants are reported herein. Additional single nucleotide polymorphisms are reported in Tables 9 and 10 and may not be shown in
 30 SEQ ID NO: 1.

NUCLEIC ACIDS OF THE INVENTION

Accordingly, the invention pertains to an isolated nucleic acid molecule comprising the human PDE4D gene having at least one nucleotide alteration and correlated with incidence of stroke. The term, "PDE4D or variant PDE4D", as used
5 herein, refers to an isolated nucleic acid molecule on chromosome 5q12 having at least one altered nucleotide that is associated with a susceptibility to a number of stroke phenotypes, and also to a portion or fragment of the isolated nucleic acid molecule (e.g., cDNA or the gene) that encodes PDE4D polypeptide (e.g., the polypeptide having SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, optionally
10 comprising at least one SNP as set forth in Tables 9 and 10, or another splicing variant of a PDE4D polypeptide). In a preferred embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:1 (shown in Appendix I) or the complement thereof. In another embodiment, the isolated nucleic acid molecule comprises the sequence of SEQ ID NO: 1 or the complement of SEQ ID NO: 1, except that one or
15 more single nucleotide polymorphisms as shown in Tables 9 and 10 are also present. In another embodiment, the isolated nucleic acid molecules comprises exon 4D6, 4D7-1, 4D7-2, 4D7-3 and 4D8.

The isolated nucleic acid molecules of the present invention can be RNA, for example, mRNA, or DNA, such as cDNA and genomic DNA. DNA molecules can
20 be double-stranded or single-stranded; single stranded RNA or DNA can be either the coding, or sense, strand or the non-coding, or antisense, strand. The nucleic acid molecule can include all or a portion of the coding sequence of the gene and can further comprise additional non-coding sequences such as introns and non-coding 3' and 5' sequences (including regulatory sequences, for example). Additionally, the
25 nucleic acid molecule can be fused to a marker sequence, for example, a sequence that encodes a polypeptide to assist in isolation or purification of the polypeptide. Such sequences include, but are not limited to, those which encode a glutathione-S-transferase (GST) fusion protein and those which encode a hemagglutinin A (HA) polypeptide marker from influenza.

30 An "isolated" nucleic acid molecule, as used herein, is one that is separated from nucleic acids which normally flank the gene or nucleotide sequence (as in

genomic sequences) and/or has been completely or partially purified from other transcribed sequences (e.g., as in an RNA library). For example, an isolated nucleic acid of the invention may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system or reagent mix. In other circumstances, the material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC.

10 Preferably, an isolated nucleic acid molecule comprises at least about 50, 80 or 90% (on a molar basis) of all macromolecular species present. With regard to genomic DNA, the term "isolated" also can refer to nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. For example, the isolated nucleic acid molecule can contain less than

15 about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotides which flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid molecule is derived.

The nucleic acid molecule can be fused to other coding or regulatory sequences and still be considered isolated. Thus, recombinant DNA contained in a

20 vector is included in the definition of "isolated" as used herein. Also, isolated nucleic acid molecules include recombinant DNA molecules in heterologous host cells, as well as partially or substantially purified DNA molecules in solution. "Isolated" nucleic acid molecules also encompass *in vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention. An isolated nucleic acid

25 molecule or nucleotide sequence can include a nucleic acid molecule or nucleotide sequence which is synthesized chemically or by recombinant means. Therefore, recombinant DNA contained in a vector are included in the definition of "isolated" as used herein. Also, isolated nucleotide sequences include recombinant DNA molecules in heterologous organisms, as well as partially or substantially purified

30 DNA molecules in solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention are also encompassed by "isolated" nucleotide

sequences. Such isolated nucleotide sequences are useful in the manufacture of the encoded polypeptide, as probes for isolating homologous sequences (e.g., from other mammalian species), for gene mapping (e.g., by *in situ* hybridization with chromosomes), or for detecting expression of the gene in tissue (e.g., human tissue),
5 such as by Northern blot analysis.

The present invention also pertains to variant nucleic acid molecules which are not necessarily found in nature but which encode a PDE4D polypeptide (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or another splicing variant of PDE4D polypeptide or polymorphic variant
10 thereof. Thus, for example, DNA molecules which comprise a sequence that is different from the naturally-occurring nucleotide sequence but which, due to the degeneracy of the genetic code, encode a PDE4D polypeptide of the present invention are also the subject of this invention. The invention also encompasses nucleotide sequences encoding portions (fragments), or encoding variant
15 polypeptides such as analogues or derivatives of the PDE4D polypeptide. Such variants can be naturally-occurring, such as in the case of allelic variation or single nucleotide polymorphisms, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion and substitution of one or more nucleotides which can
20 result in conservative or non-conservative amino acid changes, including additions and deletions. Preferably the nucleotide (and/or resultant amino acid) changes are silent or conserved; that is, they do not alter the characteristics or activity of the PDE4D polypeptide. In one preferred embodiment, the nucleotide sequences are fragments that comprise one or more polymorphic microsatellite markers. In
25 another preferred embodiment, the nucleotide sequences are fragments that comprise one or more single nucleotide polymorphisms in the PDE4D gene.

Other alterations of the nucleic acid molecules of the invention can include, for example, labeling, methylation, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates,
30 carbamates), charged linkages (e.g., phosphorothioates, phosphorodithioates), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen),

chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids). Also included are synthetic molecules that mimic nucleic acid molecules in the ability to bind to a designated sequences via hydrogen bonding and other chemical interactions. Such molecules include, for example, those in which peptide linkages
5 substitute for phosphate linkages in the backbone of the molecule.

The invention also pertains to nucleic acid molecules which hybridize under high stringency hybridization conditions, such as for selective hybridization, to a nucleotide sequence described herein (e.g., nucleic acid molecules which specifically hybridize to a nucleotide sequence encoding polypeptides described
10 herein, and, optionally, have an activity of the polypeptide). In one embodiment, the invention includes variants described herein which hybridize under high stringency hybridization conditions (e.g., for selective hybridization) to a nucleotide sequence comprising a nucleotide sequence selected from SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10 or the
15 complement thereof. In another embodiment, the invention includes variants described herein which hybridize under high stringency hybridization conditions (e.g., for selective hybridization) to a nucleotide sequence encoding an amino acid sequence selected from SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14 or polymorphic variant thereof. In a preferred embodiment, the variant which
20 hybridizes under high stringency hybridizations has an activity of PDE4D.

Such nucleic acid molecules can be detected and/or isolated by specific hybridization (e.g., under high stringency conditions). "Specific hybridization," as used herein, refers to the ability of a first nucleic acid to hybridize to a second nucleic acid in a manner such that the first nucleic acid does not hybridize to any
25 nucleic acid other than to the second nucleic acid (e.g., when the first nucleic acid has a higher similarity to the second nucleic acid than to any other nucleic acid in a sample wherein the hybridization is to be performed). "Stringency conditions" for hybridization is a term of art which refers to the incubation and wash conditions, e.g., conditions of temperature and buffer concentration, which permit hybridization
30 of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (i.e., 100%) complementary to the second, or the first and second may

share some degree of complementarity which is less than perfect (e.g., 70%, 75%, 85%, 95%). For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions", "moderate stringency conditions" and "low stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 and pages 6.3.1-6.3.6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons, (1998), the entire teachings of which are incorporated by reference herein). The exact conditions which determine the stringency of hybridization depend not only on ionic strength (e.g., 0.2XSSC, 0.1XSSC), temperature (e.g., room temperature, 42°C, 68°C) and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, equivalent conditions can be determined by varying one or more of these parameters while maintaining a similar degree of identity or similarity between the two nucleic acid molecules. Typically, conditions are used such that sequences at least about 60%, at least about 70%, at least about 80%, at least about 90% or at least about 95% or more identical to each other remain hybridized to one another. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize (e.g., selectively) with the most similar sequences in the sample can be determined.

Exemplary conditions are described in Krause, M.H. and S.A. Aaronson, *Methods in Enzymology*, 200:546-556 (1991). Also, in, Ausubel, *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons, (1998), which describes the determination of washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, starting from the lowest temperature at which only homologous hybridization occurs, each °C by

which the final wash temperature is reduced (holding SSC concentration constant) allows an increase by 1% in the maximum extent of mismatching among the sequences that hybridize. Generally, doubling the concentration of SSC results in an increase in T_m of $\sim 17^\circ\text{C}$. Using these guidelines, the washing temperature can be
5 determined empirically for high, moderate or low stringency, depending on the level of mismatch sought.

For example, a low stringency wash can comprise washing in a solution containing 0.2XSSC/0.1% SDS for 10 min at room temperature; a moderate stringency wash can comprise washing in a prewarmed solution (42°C) solution
10 containing 0.2XSSC/0.1% SDS for 15 min at 42°C ; and a high stringency wash can comprise washing in prewarmed (68°C) solution containing 0.1XSSC/0.1%SDS for 15 min at 68°C . Furthermore, washes can be performed repeatedly or sequentially to obtain a desired result as known in the art. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art,
15 while maintaining a similar degree of identity or similarity between the target nucleic acid molecule and the primer or probe used.

The percent identity of two nucleotide or amino acid sequences can be determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first sequence). The nucleotides or amino
20 acids at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions x 100). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 60%, and even more
25 preferably at least 70%, 80%, 90% or 95% of the length of the reference sequence. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:5873-5877 (1993). Such an algorithm is incorporated into the
30 NBLAST and XBLAST programs (version 2.0) as described in Altschul *et al.*, *Nucleic Acids Res.*, 25:389-3402 (1997). When utilizing BLAST and Gapped

BLAST programs, the default parameters of the respective programs (e.g., NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. In one embodiment, parameters for sequence comparison can be set at score=100, wordlength=12, or can be varied (e.g., W=5 or W=20).

5 Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120
10 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti (1994) *Comput. Appl. Biosci.*, 10:3-5; and FASTA described in Pearson and Lipman (1988) *PNAS*, 85:2444-8.

15 In another embodiment, the percent identity between two amino acid sequences can be accomplished using the GAP program in the CGC software package (available at <http://www.cgc.com>) using either a Blossom 63 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. In yet another embodiment, the percent identity between two nucleic acid
20 sequences can be accomplished using the GAP program in the GCG software package (available at <http://www.accelrys.com>), using a gap weight of 50 and a length weight of 3.

 The present invention also provides isolated nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a
25 nucleotide sequence comprising a nucleotide sequence selected from SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10 and the complement thereof, and also provides isolated nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a nucleotide sequence encoding an amino acid sequence selected from SEQ ID
30 NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or polymorphic variant thereof. The nucleic acid fragments of the invention are at least about 15, preferably at least about 18, 20,

23 or 25 nucleotides, and can be 30, 40, 50, 100, 200 or more nucleotides in length. Longer fragments, for example, 30 or more nucleotides in length, which encode antigenic polypeptides described herein are particularly useful, such as for the generation of antibodies as described below.

5 In a related aspect, the nucleic acid fragments of the invention are used as probes or primers in assays such as those described herein. "Probes" or "primers" are oligonucleotides that hybridize in a base-specific manner to a complementary strand of nucleic acid molecules. Such probes and primers include polypeptide nucleic acids, as described in Nielsen *et al.*, *Science*, 254, 1497-1500 (1991).

10 Typically, a probe or primer comprises a region of nucleotide sequence that hybridizes to at least about 15, typically about 20-25, and more typically about 40, 50 or 75, consecutive nucleotides of a nucleic acid molecule comprising a contiguous nucleotide sequence selected from: SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Tables 9 and 10, the complement
15 thereof, or a sequence encoding an amino acid sequence selected from SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or polymorphic variant thereof. In preferred embodiments, a probe or primer comprises 100 or fewer nucleotides, preferably from 6 to 50 nucleotides, preferably from 12 to 30 nucleotides. In other embodiments, the probe or primer is at least 70% identical to the contiguous
20 nucleotide sequence or to the complement of the contiguous nucleotide sequence, preferably at least 80% identical, more preferably at least 90% identical, even more preferably at least 95% identical, or even capable of selectively hybridizing to the contiguous nucleotide sequence or to the complement of the contiguous nucleotide sequence. Often, the probe or primer further comprises a label, *e.g.*, radioisotope,
25 fluorescent compound, enzyme, or enzyme co-factor.

The nucleic acid molecules of the invention such as those described above can be identified and isolated using standard molecular biology techniques and the sequence information provided herein. For example, nucleic acid molecules can be amplified and isolated by the polymerase chain reaction using synthetic
30 oligonucleotide primers designed based on one or more of the sequences provided in SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in

Tables 9 and 10, and/or the complement thereof, or designed based on nucleotides based on sequences encoding one or more of the amino acid sequences provided herein. See generally *PCR Technology: Principles and Applications for DNA Amplification* (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); *PCR Protocols: A Guide to Methods and Applications* (Eds. Innis, *et al.*, Academic Press, San Diego, CA, 1990); Mattila *et al.*, *Nucleic Acids Res.*, 19:4967 (1991); Eckert *et al.*, *PCR Methods and Applications*, 1:17 (1991); PCR (eds. McPherson *et al.*, IRL Press, Oxford); and U.S. Patent 4,683,202. The nucleic acid molecules can be amplified using cDNA, mRNA or genomic DNA as a template, cloned into an appropriate vector and characterized by DNA sequence analysis.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, *Genomics*, 4:560 (1989), Landegren *et al.*, *Science*, 241:1077 (1988), transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:1173 (1989)), and self-sustained sequence replication (Guatelli *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

The amplified DNA can be radiolabelled and used as a probe for screening a cDNA library derived from human cells, mRNA in zap express, ZIPLOX or other suitable vector. Corresponding clones can be isolated, DNA can be obtained following *in vivo* excision, and the cloned insert can be sequenced in either or both orientations by art recognized methods to identify the correct reading frame encoding a polypeptide of the appropriate molecular weight. For example, the direct analysis of the nucleotide sequence of nucleic acid molecules of the present invention can be accomplished using well-known methods that are commercially available. See, for example, Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind *et al.*, *Recombinant DNA Laboratory Manual*, (Acad. Press, 1988)). Using these or similar methods, the polypeptide and the DNA encoding the polypeptide can be isolated, sequenced and further characterized.

Antisense nucleic acid molecules of the invention can be designed using the nucleotide sequences of SEQ ID NO: 1 and/or the complement of SEQ ID NO: 1, and/or a portion of SEQ ID NO:1 or the complement of SEQ ID NO:1 and/or a sequence encoding the amino acid sequences or SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and/or 14, or encoding a portion of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and/or 14, (wherein any one of these may optionally comprise at least one polymorphism as shown in Tables 9 and 10) and constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid molecule (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the antisense nucleic acid molecule can be produced biologically using an expression vector into which a nucleic acid molecule has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid molecule will be of an antisense orientation to a target nucleic acid of interest).

In general, the isolated nucleic acid sequences of the invention can be used as molecular weight markers on Southern gels, and as chromosome markers which are labeled to map related gene positions. The nucleic acid sequences can also be used to compare with endogenous DNA sequences in patients to identify genetic disorders (*e.g.*, a predisposition for or susceptibility to stroke), and as probes, such as to hybridize and discover related DNA sequences or to subtract out known sequences from a sample. The nucleic acid sequences can further be used to derive primers for genetic fingerprinting, to raise anti-polypeptide antibodies using DNA immunization techniques, and as an antigen to raise anti-DNA antibodies or elicit immune responses. Portions or fragments of the nucleotide sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions

associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Additionally, the nucleotide sequences of the invention can be used to identify and express recombinant polypeptides for analysis, characterization or therapeutic use, or
5 as markers for tissues in which the corresponding polypeptide is expressed, either constitutively, during tissue differentiation, or in diseased states. The nucleic acid sequences can additionally be used as reagents in the screening and/or diagnostic assays described herein, and can also be included as components of kits (e.g., reagent kits) for use in the screening and/or diagnostic assays described herein.

10 Another aspect of the invention pertains to nucleic acid constructs containing a nucleic acid molecule selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 and the complement thereof (or a portion thereof). Yet another aspect of the invention pertains to nucleic acid constructs containing a nucleic acid molecule encoding the
15 amino acid sequence of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14 or polymorphic variant thereof. The constructs comprise a vector (e.g., an expression vector) into which a sequence of the invention has been inserted in a sense or antisense orientation. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.
20 One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of
25 replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in
30 recombinant DNA techniques are often in the form of plasmids. However, the invention is intended to include such other forms of expression vectors, such as viral

vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses) that serve equivalent functions.

Preferred recombinant expression vectors of the invention comprise a nucleic acid molecule of the invention in a form suitable for expression of the nucleic acid molecule in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably or operatively linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed and the level of expression of polypeptide desired. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides, including fusion polypeptides, encoded by nucleic acid molecules as described herein.

The recombinant expression vectors of the invention can be designed for expression of a polypeptide of the invention in prokaryotic or eukaryotic cells, e.g., bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a nucleic acid molecule of the invention can be expressed in bacterial cells (*e.g.*, *E. coli*), insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing a foreign nucleic acid molecule (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector as the nucleic acid molecule of the invention or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid molecule can be identified by drug

selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a polypeptide of the invention.

5 Accordingly, the invention further provides methods for producing a polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another embodiment, the method further
10 comprises isolating the polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which a nucleic acid molecule of the invention has been introduced (*e.g.*, an exogenous PDE4D gene, or an exogenous
15 nucleic acid encoding PDE4D polypeptide). Such host cells can then be used to create non-human transgenic animals in which exogenous nucleotide sequences have been introduced into the genome or homologous recombinant animals in which endogenous nucleotide sequences have been altered. Such animals are useful for studying the function and/or activity of the nucleotide sequence and polypeptide
20 encoded by the sequence and for identifying and/or evaluating modulators of their activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens and amphibians. A
25 transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, an "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably
30 a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule

introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, 5 U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology*, 10 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169. Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature*, 385:810-813 and PCT Publication Nos. WO 97/07668 and WO 97/07669.

POLYPEPTIDES OF THE INVENTION

15 The present invention also pertains to isolated polypeptides encoded by PDE4D ("PDE4D polypeptides") and fragments and variants thereof, as well as polypeptides encoded by nucleotide sequences described herein (*e.g.*, other splicing variants). The term "polypeptide" refers to a polymer of amino acids, and not to a specific length; thus, peptides, oligopeptides and proteins are included within the 20 definition of a polypeptide. As used herein, a polypeptide is said to be "isolated" or "purified" when it is substantially free of cellular material when it is isolated from recombinant and non-recombinant cells, or free of chemical precursors or other chemicals when it is chemically synthesized. A polypeptide, however, can be joined to another polypeptide with which it is not normally associated in a cell (*e.g.*, in a 25 "fusion protein") and still be "isolated" or "purified."

The polypeptides of the invention can be purified to homogeneity. It is understood, however, that preparations in which the polypeptide is not purified to homogeneity are useful. The critical feature is that the preparation allows for the desired function of the polypeptide, even in the presence of considerable amounts of 30 other components. Thus, the invention encompasses various degrees of purity. In

one embodiment, the language "substantially free of cellular material" includes preparations of the polypeptide having less than about 30% (by dry weight) other proteins (*i.e.*, contaminating protein), less than about 20% other proteins, less than about 10% other proteins, or less than about 5% other proteins.

- 5 When a polypeptide is recombinantly produced, it can also be substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, less than about 10%, or less than about 5% of the volume of the polypeptide preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide in which it is separated from chemical precursors or
- 10 other chemicals that are involved in its synthesis. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide having less than about 30% (by dry weight) chemical precursors or other chemicals, less than about 20% chemical precursors or other chemicals, less than about 10% chemical precursors or other chemicals, or less than about 5%
- 15 chemical precursors or other chemicals.

- In one embodiment, a polypeptide of the invention comprises an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 and complements and portions
- 20 thereof, *e.g.*, SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or a portion or polymorphic variant thereof. However, the polypeptides of the invention also encompass fragment and sequence variants. Variants include a substantially homologous polypeptide encoded by the same genetic locus in an organism, *i.e.*, an allelic variant, as well as other splicing variants. Variants also encompass
- 25 polypeptides derived from other genetic loci in an organism, but having substantial homology to a polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 and complements and portions thereof, or having substantial homology to a polypeptide
- 30 encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of nucleotide sequences encoding SEQ ID NO: 2, 3, 4, 5, 6, 7,

8, 9, 10, 12 or 14, or polymorphic variants thereof. Variants also include polypeptides substantially homologous or identical to these polypeptides but derived from another organism, *i.e.*, an ortholog. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by chemical synthesis. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by recombinant methods.

As used herein, two polypeptides (or a region of the polypeptides) are substantially homologous or identical when the amino acid sequences are at least about 45-55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically greater than about 90% or more homologous or identical. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid molecule hybridizing to SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10, or portion thereof, under stringent conditions as more particularly described above, or will be encoded by a nucleic acid molecule hybridizing to a nucleic acid sequence encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, portion thereof or polymorphic variant thereof, under stringent conditions as more particularly described thereof.

To determine the percent homology or identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of one polypeptide or nucleic acid molecule for optimal alignment with the other polypeptide or nucleic acid molecule). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence, then the molecules are homologous at that position. As used herein, amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity". The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, percent homology equals the number of identical positions/total number of positions times 100).

The invention also encompasses polypeptides having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions performed by a polypeptide encoded by a nucleic acid molecule of the invention. Similarity is determined by conserved amino acid substitution. Such
5 substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Conservative substitutions are likely to be phenotypically silent. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic
10 residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe and Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie *et al.*, *Science* 247:1306-1310 (1990).

A variant polypeptide can differ in amino acid sequence by one or more
15 substitutions, deletions, insertions, inversions, fusions, and truncations or a combination of any of these. Further, variant polypeptides can be fully functional or can lack function in one or more activities. Fully functional variants typically contain only conservative variation or variation in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar
20 amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

25 Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham *et al.*, *Science*, 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity *in vitro*, or *in vitro*
30 proliferative activity. Sites that are critical for polypeptide activity can also be determined by structural analysis such as crystallization, nuclear magnetic resonance

or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.*, 224:899-904 (1992); de Vos *et al.*, *Science*, 255:306-312 (1992)).

The invention also includes polypeptide fragments of the polypeptides of the invention. Fragments can be derived from a polypeptide encoded by a nucleic acid molecule comprising SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 or a portion thereof and the complements thereof (e.g., SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or other splicing variants). However, the invention also encompasses fragments of the variants of the polypeptides described herein. As used herein, a fragment comprises at least 6 contiguous amino acids. Useful fragments include those that retain one or more of the biological activities of the polypeptide as well as fragments that can be used as an immunogen to generate polypeptide-specific antibodies.

Biologically active fragments (peptides which are, for example, 6, 9, 12, 15, 16, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) can comprise a domain, segment, or motif that has been identified by analysis of the polypeptide sequence using well-known methods, e.g., signal peptides, extracellular domains, one or more transmembrane segments or loops, ligand binding regions, zinc finger domains, DNA binding domains, acylation sites, glycosylation sites, or phosphorylation sites.

Fragments can be discrete (not fused to other amino acids or polypeptides) or can be within a larger polypeptide. Further, several fragments can be comprised within a single larger polypeptide. In one embodiment a fragment designed for expression in a host can have heterologous pre- and pro-polypeptide regions fused to the amino terminus of the polypeptide fragment and an additional region fused to the carboxyl terminus of the fragment.

The invention thus provides chimeric or fusion polypeptides. These comprise a polypeptide of the invention operatively linked to a heterologous protein or polypeptide having an amino acid sequence not substantially homologous to the polypeptide. "Operatively linked" indicates that the polypeptide and the heterologous protein are fused in-frame. The heterologous protein can be fused to the N-terminus or C-terminus of the polypeptide. In one embodiment the fusion

polypeptide does not affect function of the polypeptide *per se*. For example, the fusion polypeptide can be a GST-fusion polypeptide in which the polypeptide sequences are fused to the C-terminus of the GST sequences. Other types of fusion polypeptides include, but are not limited to, enzymatic fusion polypeptides, for example β -galactosidase fusions, yeast two-hybrid GAL fusions, poly-His fusions and Ig fusions. Such fusion polypeptides, particularly poly-His fusions, can facilitate the purification of recombinant polypeptide. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of a polypeptide can be increased by using a heterologous signal sequence. Therefore, in another embodiment, the fusion polypeptide contains a heterologous signal sequence at its N-terminus.

EP-A-O 464 533 discloses fusion proteins comprising various portions of immunoglobulin constant regions. The Fc is useful in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). In drug discovery, for example, human proteins have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists. Bennett *et al.*, *Journal of Molecular Recognition*, 8:52-58 (1995) and Johanson *et al.*, *The Journal of Biological Chemistry*, 270,16:9459-9471 (1995). Thus, this invention also encompasses soluble fusion polypeptides containing a polypeptide of the invention and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclass (IgG, IgM, IgA, IgE).

A chimeric or fusion polypeptide can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of nucleic acid fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive nucleic acid fragments which can subsequently be annealed and re-amplified to generate a chimeric nucleic acid sequence (see Ausubel *et al.*, *Current Protocols in Molecular Biology*, 1992). Moreover, many expression vectors are commercially available that already encode a

fusion moiety (*e.g.*, a GST protein). A nucleic acid molecule encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the polypeptide.

The isolated polypeptide can be purified from cells that naturally express it, 5 purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods. In one embodiment, the polypeptide is produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the polypeptide is cloned into an expression vector, the expression vector introduced into a host cell and the polypeptide expressed in the host cell. The 10 polypeptide can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques.

In general, polypeptides of the present invention can be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using art-recognized methods. The polypeptides of the present invention can be 15 used to raise antibodies or to elicit an immune response. The polypeptides can also be used as a reagent, *e.g.*, a labeled reagent, in assays to quantitatively determine levels of the polypeptide or a molecule to which it binds (*e.g.*, a receptor or a ligand) in biological fluids. The polypeptides can also be used as markers for cells or tissues in which the corresponding polypeptide is preferentially expressed, either 20 constitutively, during tissue differentiation, or in a diseased state. The polypeptides can be used to isolate a corresponding binding agent, *e.g.*, receptor or ligand, such as, for example, in an interaction trap assay, and to screen for peptide or small molecule antagonists or agonists of the binding interaction.

ANTIBODIES OF THE INVENTION

25 Polyclonal and/or monoclonal antibodies that specifically bind one form of the gene product but not to the other form of the gene product are also provided. Antibodies are also provided that bind a portion of either the variant or the reference gene product that contains the polymorphic site or sites. The invention provides antibodies to the polypeptides and polypeptide fragments of the invention, *e.g.*, 30 having an amino acid sequence encoded by SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12

or 14, or a portion thereof, or having an amino acid sequence encoded by a nucleic acid molecule comprising all or a portion of SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 (e.g., SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or another splicing variant or portion thereof). The

5 term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. A molecule that specifically binds to a polypeptide of the invention is a molecule that binds to that polypeptide or a fragment thereof, but does not substantially bind other molecules in

10 a sample, *e.g.*, a biological sample, which naturally contains the polypeptide. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind to a polypeptide of the invention. The term "monoclonal

15 antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a polypeptide of the invention. A monoclonal antibody composition thus typically displays a single binding affinity for a particular polypeptide of the invention with which it

20 immunoreacts.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a desired immunogen, *e.g.*, polypeptide of the invention or fragment thereof. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent

25 assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules directed against the polypeptide can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells

30 can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler

and Milstein (1975) *Nature*, 256:495-497, the human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today*, 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well
5 known (see generally *Current Protocols in Immunology* (1994) Coligan *et al.* (eds.) John Wiley & Sons, Inc., New York, NY). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a
10 monoclonal antibody that binds a polypeptide of the invention.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody to a polypeptide of the invention (see, *e.g.*, *Current Protocols in Immunology*, *supra*; Galfre *et al.* (1977) *Nature*, 266:55052; R.H. Kenneth, in
15 *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner (1981) *Yale J. Biol. Med.*, 54:387-402. Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful.

Alternative to preparing monoclonal antibody-secreting hybridomas, a
20 monoclonal antibody to a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with the polypeptide to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage*
25 *Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™* Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791;
30 PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT

Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology*, 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas*, 3:81-85; Huse *et al.* (1989) *Science*, 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.*, 12:725-734.

Additionally, recombinant antibodies, such as chimeric and humanized
5 monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art.

In general, antibodies of the invention (*e.g.*, a monoclonal antibody) can be
10 used to isolate a polypeptide of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. A polypeptide-specific antibody can facilitate the purification of natural polypeptide from cells and of recombinantly produced polypeptide expressed in host cells. Moreover, an antibody specific for a polypeptide of the invention can be used to detect the polypeptide (*e.g.*, in a cellular
15 lysate, cell supernatant, or tissue sample) in order to evaluate the abundance and pattern of expression of the polypeptide. Antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of
20 detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples
25 of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

DIAGNOSTIC AND SCREENING ASSAYS OF THE INVENTION

The present invention also pertains to a method of diagnosing or aiding in the diagnosis of stroke associated with the presence of the PDE4D gene or gene product in an individual. Diagnostic assays can be designed for assessing PDE4D gene
5 expression, or for assessing activity of PDE4D polypeptides of the invention. In one embodiment, the assays are used in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with stroke, or is at risk for (has a predisposition for or a susceptibility to) developing stroke. The invention also provides for prognostic (or predictive) assays for
10 determining whether an individual is susceptible to developing stroke. For example, mutations in the gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of symptoms associated with stroke. Another aspect of the invention pertains to assays for monitoring the influence of agents (*e.g.*, drugs,
15 compounds or other agents) on the gene expression or activity of polypeptides of the invention, as well as to assays for identifying agents which bind to PDE4D polypeptides. These and other assays and agents are described in further detail in the following sections.

DIAGNOSTIC ASSAYS

20 The nucleic acids, probes, primers, polypeptides and antibodies described herein can be used in methods of diagnosis of a susceptibility to stroke, as well as in kits useful for diagnosis of a susceptibility to stroke.

In one embodiment of the invention, diagnosis of a susceptibility to stroke is made by detecting a polymorphism in PDE4D as described herein. The
25 polymorphism can be a mutation in PDE4D, such as the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift mutation; the change of at least one nucleotide, resulting in a change in the encoded amino acid; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the deletion of several nucleotides, resulting in a deletion of one or more
30 amino acids encoded by the nucleotides; the insertion of one or several nucleotides,

such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such mutation may be present in a single gene. Such sequence changes cause a mutation in the polypeptide encoded by a PDE4D gene. For example, if the mutation is a frame shift mutation, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated polypeptide. Alternatively, a polymorphism associated with a susceptibility to stroke can be a synonymous mutation in one or more nucleotides (i.e., a mutation that does not result in a change in the polypeptide encoded by a PDE4D gene). Such a polymorphism may alter splicing sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of the gene. A PDE4D gene that has any of the mutations described above is referred to herein as a "mutant gene."

In a first method of diagnosing a susceptibility to stroke, hybridization methods, such as Southern analysis, Northern analysis, or *in situ* hybridizations, can be used (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, including all supplements through 1999). For example, a biological sample from a test subject (a "test sample") of genomic DNA, RNA, or cDNA, is obtained from an individual suspected of having, being susceptible to or predisposed for, or carrying a defect for, stroke (the "test individual"). The individual can be an adult, child, or fetus. The test sample can be from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or other organs. A test sample of DNA from fetal cells or tissue can be obtained by appropriate methods, such as by amniocentesis or chorionic villus sampling. The DNA, RNA, or cDNA sample is then examined to determine whether a polymorphism in *PDE4D* is present, and/or to determine which splicing variant(s) encoded by PDE4D is present. The presence of the polymorphism or splicing variant(s) can be indicated by hybridization of the gene in the genomic DNA, RNA, or cDNA to a nucleic acid probe. A "nucleic acid

probe", as used herein, can be a DNA probe or an RNA probe; the nucleic acid probe can contain at least one polymorphism in PDE4D or contains a nucleic acid encoding a particular splicing variant of PDE4D. The probe can be any of the nucleic acid molecules described above (e.g., the gene, a fragment, a vector
5 comprising the gene, a probe or primer, etc.).

To diagnose a susceptibility to stroke, a hybridization sample is formed by contacting the test sample containing PDE4D, with at least one nucleic acid probe. A preferred probe for detecting mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA sequences described
10 herein. The nucleic acid probe can be, for example, a full-length nucleic acid molecule, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to appropriate mRNA or genomic DNA. For example, the nucleic acid probe can be all or a portion of SEQ ID NO: 1 which may optionally
15 comprise at least one polymorphism shown in Tables 9 and 10, or the complement thereof, or a portion thereof; or can be a nucleic acid encoding a portion of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14. Other suitable probes for use in the diagnostic assays of the invention are described above (see e.g., probes and primers discussed under the heading, "Nucleic Acids of the Invention").

20 The hybridization sample is maintained under conditions which are sufficient to allow specific hybridization of the nucleic acid probe to PDE4D. "Specific hybridization", as used herein, indicates exact hybridization (e.g., with no mismatches). Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, for example, as described above. In a
25 particularly preferred embodiment, the hybridization conditions for specific hybridization are high stringency.

Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the nucleic acid probe and PDE4D in the test sample, then PDE4D has the polymorphism, or is the splicing variant, that is present
30 in the nucleic acid probe. More than one nucleic acid probe can also be used concurrently in this method. Specific hybridization of any one of the nucleic acid

probes is indicative of a polymorphism in PDE4D, or of the presence of a particular splicing variant encoding PDE4D and is therefore diagnostic for a susceptibility to stroke.

In Northern analysis (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, *supra*) the hybridization methods described above are used to identify the presence of a polymorphism or a particular splicing variant, associated with a susceptibility to stroke. For Northern analysis, a test sample of RNA is obtained from the individual by appropriate means. Specific hybridization of a nucleic acid probe, as described above, to RNA from the individual is indicative of a polymorphism in PDE4D, or of the presence of a particular splicing variant encoded by PDE4D, and is therefore diagnostic for a susceptibility to stroke.

For representative examples of use of nucleic acid probes, see, for example, U.S. Patents No. 5,288,611 and 4,851,330.

Alternatively, a peptide nucleic acid (PNA) probe can be used instead of a nucleic acid probe in the hybridization methods described above. PNA is a DNA mimic having a peptide-like, inorganic backbone, such as N-(2-aminoethyl)glycine units, with an organic base (A, G, C, T or U) attached to the glycine nitrogen via a methylene carbonyl linker (see, for example, Nielsen, P.E. *et al.*, *Bioconjugate Chemistry*, 1994, 5, American Chemical Society, p. 1 (1994)). The PNA probe can be designed to specifically hybridize to a gene having a polymorphism associated with a susceptibility to stroke. Hybridization of the PNA probe to PDE4D is diagnostic for a susceptibility to stroke.

In another method of the invention, mutation analysis by restriction digestion can be used to detect a mutant gene, or genes containing a polymorphism(s), if the mutation or polymorphism in the gene results in the creation or elimination of a restriction site. A test sample containing genomic DNA is obtained from the individual. Polymerase chain reaction (PCR) can be used to amplify PDE4D (and, if necessary, the flanking sequences) in the test sample of genomic DNA from the test individual. RFLP analysis is conducted as described (see Current Protocols in Molecular Biology, *supra*). The digestion pattern of the relevant DNA fragment

indicates the presence or absence of the mutation or polymorphism in PDE4D, and therefore indicates the presence or absence of this susceptibility to stroke.

Sequence analysis can also be used to detect specific polymorphisms in PDE4D. A test sample of DNA or RNA is obtained from the test individual. PCR
5 or other appropriate methods can be used to amplify the gene, and/or its flanking sequences, if desired. The sequence of PDE4D, or a fragment of the gene, or cDNA, or fragment of the cDNA, or mRNA, or fragment of the mRNA, is determined, using standard methods. The sequence of the gene, gene fragment, cDNA, cDNA
10 sequence of the gene, cDNA (e.g., SEQ ID NO:1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10, or a nucleic acid sequence encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or a fragment thereof) or mRNA, as appropriate. The presence of a polymorphism in PDE4D indicates that the individual has a susceptibility to stroke.

15 Allele-specific oligonucleotides can also be used to detect the presence of a polymorphism in PDE4D, through the use of dot-blot hybridization of amplified oligonucleotides with allele-specific oligonucleotide (ASO) probes (see, for example, Saiki, R. *et al.*, (1986), *Nature (London)* 324:163-166). An "allele-specific oligonucleotide" (also referred to herein as an "allele-specific oligonucleotide
20 probe") is an oligonucleotide of approximately 10-50 base pairs, preferably approximately 15-30 base pairs, that specifically hybridizes to PDE4D, and that contains a polymorphism associated with a susceptibility to stroke. An allele-specific oligonucleotide probe that is specific for particular polymorphisms in PDE4D can be prepared, using standard methods (see Current Protocols in
25 Molecular Biology, *supra*). To identify polymorphisms in the gene that are associated with a susceptibility to stroke, a test sample of DNA is obtained from the individual. PCR can be used to amplify all or a fragment of PDE4D, and its flanking sequences. The DNA containing the amplified PDE4D (or fragment of the gene) is dot-blotted, using standard methods (see Current Protocols in Molecular
30 Biology, *supra*), and the blot is contacted with the oligonucleotide probe. The presence of specific hybridization of the probe to the amplified PDE4D is then

detected. Specific hybridization of an allele-specific oligonucleotide probe to DNA from the individual is indicative of a polymorphism in PDE4D, and is therefore indicative of a susceptibility to stroke.

In another embodiment, arrays of oligonucleotide probes that are
5 complementary to target nucleic acid sequence segments from an individual, can be used to identify polymorphisms in PDE4D. For example, in one embodiment, an oligonucleotide array can be used. Oligonucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These oligonucleotide arrays, also described
10 as "Genechips.TM.," have been generally described in the art, for example, U.S. Pat. No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092. These arrays can generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods. See Fodor *et al.*,
15 *Science*, 251:767-777 (1991), Pirrung *et al.*, U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor *et al.*, PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, the entire teachings of each of which are incorporated by reference herein. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. Nos. 5,384,261, the
20 entire teachings of which are incorporated by reference herein.

Once an oligonucleotide array is prepared, a nucleic acid of interest is hybridized with the array and scanned for polymorphisms. Hybridization and scanning are generally carried out by methods described herein and also in, e.g., Published PCT Application Nos. WO 92/10092 and WO 95/11995, and U.S. Pat.
25 No. 5,424,186, the entire teachings of which are incorporated by reference herein. In brief, a target nucleic acid sequence which includes one or more previously identified polymorphic markers is amplified by well known amplification techniques, e.g., PCR. Typically, this involves the use of primer sequences that are complementary to the two strands of the target sequence both upstream and
30 downstream from the polymorphism. Asymmetric PCR techniques may also be used. Amplified target, generally incorporating a label, is then hybridized with the

array under appropriate conditions. Upon completion of hybridization and washing of the array, the array is scanned to determine the position on the array to which the target sequence hybridizes. The hybridization data obtained from the scan is typically in the form of fluorescence intensities as a function of location on the array.

5 Although primarily described in terms of a single detection block, e.g., for detection of a single polymorphism, arrays can include multiple detection blocks, and thus be capable of analyzing multiple, specific polymorphisms. In alternate arrangements, it will generally be understood that detection blocks may be grouped within a single array or in multiple, separate arrays so that varying, optimal
10 conditions may be used during the hybridization of the target to the array. For example, it may often be desirable to provide for the detection of those polymorphisms that fall within G-C rich stretches of a genomic sequence, separately from those falling in A-T rich segments. This allows for the separate optimization of hybridization conditions for each situation.

15 Additional description of use of oligonucleotide arrays for detection of polymorphisms can be found, for example, in U.S. Patents 5,858,659 and 5,837,832, the entire teachings of which are incorporated by reference herein.

Other methods of nucleic acid analysis can be used to detect polymorphisms in PDE4D or splicing variants encoding by PDE4D. Representative methods
20 include direct manual sequencing (Church and Gilbert, (1988), *Proc. Natl. Acad. Sci. USA* 81:1991-1995; Sanger, F. *et al.* (1977) *Proc. Natl. Acad. Sci.* 74:5463-5467; Beavis *et al.* U.S. Pat. No. 5,288,644); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP); clamped denaturing gel electrophoresis (CDGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield,
25 V.C. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:232-236), mobility shift analysis (Orita, M. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2766-2770), restriction enzyme analysis (Flavell *et al.* (1978) *Cell* 15:25; Geever, *et al.* (1981) *Proc. Natl. Acad. Sci. USA* 78:5081); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 85:4397-4401); RNase
30 protection assays (Myers, R.M. *et al.* (1985) *Science* 230:1242); use of polypeptides

which recognize nucleotide mismatches, such as *E. coli* mutS protein; allele-specific PCR, for example.

In another embodiment of the invention, diagnosis of a susceptibility to stroke can also be made by examining expression and/or composition of an PDE4D polypeptide, by a variety of methods, including enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. A test sample from an individual is assessed for the presence of an alteration in the expression and/or an alteration in composition of the polypeptide encoded by PDE4D, or for the presence of a particular variant encoded by PDE4D. An alteration in expression of a polypeptide encoded by PDE4D can be, for example, an alteration in the quantitative polypeptide expression (i.e., the amount of polypeptide produced); an alteration in the composition of a polypeptide encoded by PDE4D is an alteration in the qualitative polypeptide expression (e.g., expression of a mutant PDE4D polypeptide or of a different splicing variant). In a preferred embodiment, diagnosis of a susceptibility to stroke is made by detecting a particular splicing variant encoded by PDE4D, or a particular pattern of splicing variants.

Both such alterations (quantitative and qualitative) can also be present. An "alteration" in the polypeptide expression or composition, as used herein, refers to an alteration in expression or composition in a test sample, as compared with the expression or composition of polypeptide by PDE4D in a control sample. A control sample is a sample that corresponds to the test sample (e.g., is from the same type of cells), and is from an individual who is not affected by stroke. An alteration in the expression or composition of the polypeptide in the test sample, as compared with the control sample, is indicative of a susceptibility to stroke. Similarly, the presence of one or more different splicing variants in the test sample, or the presence of significantly different amounts of different splicing variants in the test sample, as compared with the control sample, is indicative of a susceptibility to stroke. Various means of examining expression or composition of the polypeptide encoded by PDE4D can be used, including spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and immunoassays (e.g., David *et al.*, U.S. Pat. No. 4,376,110) such as immunoblotting (see also Current Protocols in Molecular Biology,

particularly chapter 10). For example, in one embodiment, an antibody capable of binding to the polypeptide (e.g., as described above), preferably an antibody with a detectable label, can be used. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

Western blotting analysis, using an antibody as described above that specifically binds to a polypeptide encoded by a mutant PDE4D, or an antibody that specifically binds to a polypeptide encoded by a non-mutant gene, or an antibody that specifically binds to a particular splicing variant encoded by PDE4D, can be used to identify the presence in a test sample of a particular splicing variant or of a polypeptide encoded by a polymorphic or mutant PDE4D, or the absence in a test sample of a particular splicing variant or of a polypeptide encoded by a non-polymorphic or non-mutant gene. The presence of a polypeptide encoded by a polymorphic or mutant gene, or the absence of a polypeptide encoded by a non-polymorphic or non-mutant gene, is diagnostic for a susceptibility to stroke, as is the presence (or absence) of particular splicing variants encoded by the PDE4D gene.

In one embodiment of this method, the level or amount of polypeptide encoded by PDE4D in a test sample is compared with the level or amount of the polypeptide encoded by PDE4D in a control sample. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an alteration in the expression of the polypeptide encoded by PDE4D, and is diagnostic for a susceptibility to stroke. Alternatively, the composition of the polypeptide encoded by PDE4D in a test sample is compared with the composition of the polypeptide encoded by PDE4D in a control sample

- (e.g., the presence of different splicing variants). A difference in the composition of the polypeptide in the test sample, as compared with the composition of the polypeptide in the control sample, is diagnostic for a susceptibility to stroke. In another embodiment, both the level or amount and the composition of the polypeptide can be assessed in the test sample and in the control sample. A difference in the amount or level of the polypeptide in the test sample, compared to the control sample; a difference in composition in the test sample, compared to the control sample; or both a difference in the amount or level, and a difference in the composition, is indicative of a susceptibility to stroke.
- 10 Kits (e.g., reagent kits) useful in the methods of diagnosis comprise components useful in any of the methods described herein, including for example, hybridization probes or primers as described herein (e.g., labeled probes or primers), reagents for detection of labeled molecules, restriction enzymes (e.g., for RFLP analysis), allele-specific oligonucleotides, antibodies which bind to mutant or to
- 15 non-mutant (native) PDE4D polypeptide, means for amplification of nucleic acids comprising PDE4D, or means for analyzing the nucleic acid sequence of PDE4D or for analyzing the amino acid sequence of an PDE4D polypeptide, etc.

SCREENING ASSAYS AND AGENTS IDENTIFIED THEREBY

- The invention provides methods (also referred to herein as "screening assays") for identifying the presence of a nucleotide that hybridizes to a nucleic acid of the invention, as well as for identifying the presence of a polypeptide encoded by a nucleic acid of the invention. In one embodiment, the presence (or absence) of a nucleic acid molecule of interest (e.g., a nucleic acid that has significant homology with a nucleic acid of the invention) in a sample can be assessed by contacting the
- 25 sample with a nucleic acid comprising a nucleic acid of the invention (e.g., a nucleic acid having the sequence of SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10, or the complement thereof, or a nucleic acid encoding an amino acid having the sequence of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or a fragment or variant of such nucleic acids), under
- 30 stringent conditions as described above, and then assessing the sample for the

presence (or absence) of hybridization. In a preferred embodiment, high stringency conditions are conditions appropriate for selective hybridization. In another embodiment, a sample containing the nucleic acid molecule of interest is contacted with a nucleic acid containing a contiguous nucleotide sequence (e.g., a primer or a probe as described above) that is at least partially complementary to a part of the nucleic acid molecule of interest (e.g., a PDE4D nucleic acid), and the contacted sample is assessed for the presence or absence of hybridization. In a preferred embodiment, the nucleic acid containing a contiguous nucleotide sequence is completely complementary to a part of the nucleic acid molecule of interest.

10 In any of these embodiment, all or a portion of the nucleic acid of interest can be subjected to amplification prior to performing the hybridization.

In another embodiment, the presence (or absence) of a polypeptide of interest, such as a polypeptide of the invention or a fragment or variant thereof, in a sample can be assessed by contacting the sample with an antibody that specifically hybridizes to the polypeptide of interest (e.g., an antibody such as those described above), and then assessing the sample for the presence (or absence) of binding of the antibody to the polypeptide of interest.

In another embodiment, the invention provides methods for identifying agents (e.g., fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes which alter (e.g., increase or decrease) the activity of the polypeptides described herein, or which otherwise interact with the polypeptides herein. For example, such agents can be agents which bind to polypeptides described herein (e.g., PDE4D binding agents); which have a stimulatory or inhibitory effect on, for example, activity of polypeptides of the invention; or which change (e.g., enhance or inhibit) the ability of the polypeptides of the invention to interact with PDE4D binding agents (e.g., receptors or other binding agents); or which alter posttranslational processing of the PDE4D polypeptide (e.g., agents that alter proteolytic processing to direct the polypeptide from where it is normally synthesized to another location in the cell, such as the cell surface; agents that alter proteolytic processing such that more polypeptide is released from the cell, etc.

In one embodiment, the invention provides assays for screening candidate or test agents that bind to or modulate the activity of polypeptides described herein (or biologically active portion(s) thereof), as well as agents identifiable by the assays. Test agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.*, 12:145).

In one embodiment, to identify agents which alter the activity of a PDE4D polypeptide, a cell, cell lysate, or solution containing or expressing a PDE4D polypeptide (e.g., SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or another splicing variant encoded by PDE4D), or a fragment or derivative thereof (as described above), can be contacted with an agent to be tested; alternatively, the polypeptide can be contacted directly with the agent to be tested. The level (amount) of PDE4D activity is assessed (e.g., the level (amount) of PDE4D activity is measured, either directly or indirectly), and is compared with the level of activity in a control (i.e., the level of activity of the PDE4D polypeptide or active fragment or derivative thereof in the absence of the agent to be tested). If the level of the activity in the presence of the agent differs, by an amount that is statistically significant, from the level of the activity in the absence of the agent, then the agent is an agent that alters the activity of PDE4D polypeptide. An increase in the level of PDE4D activity relative to a control, indicates that the agent is an agent that enhances (is an agonist of) PDE4D activity. Similarly, a decrease in the level of PDE4D activity relative to a control, indicates that the agent is an agent that inhibits (is an antagonist of) PDE4D activity. In another embodiment, the level of activity of a PDE4D polypeptide or derivative or fragment thereof in the presence of the agent to be tested, is compared with a control level that has previously been established. A level of the activity in the

presence of the agent that differs from the control level by an amount that is statistically significant indicates that the agent alters PDE4D activity.

The present invention also relates to an assay for identifying agents which alter the expression of the PDE4D gene (e.g., antisense nucleic acids, fusion
5 proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) which alter (e.g., increase or decrease) expression (e.g., transcription or translation) of the gene or which otherwise interact with the nucleic acids described herein, as well as agents identifiable by the assays. For example, a solution containing a nucleic acid
10 encoding PDE4D polypeptide (e.g., PDE4D gene) can be contacted with an agent to be tested. The solution can comprise, for example, cells containing the nucleic acid or cell lysate containing the nucleic acid; alternatively, the solution can be another solution which comprises elements necessary for transcription/translation of the nucleic acid. Cells not suspended in solution can also be employed, if desired. The
15 level and/or pattern of PDE4D expression (e.g., the level and/or pattern of mRNA or of protein expressed, such as the level and/or pattern of different splicing variants) is assessed, and is compared with the level and/or pattern of expression in a control (i.e., the level and/or pattern of the PDE4D expression in the absence of the agent to be tested). If the level and/or pattern in the presence of the agent differs, by an
20 amount or in a manner that is statistically significant, from the level and/or pattern in the absence of the agent, then the agent is an agent that alters the expression of PDE4D. Enhancement of PDE4D expression indicates that the agent is an agonist of PDE4D activity. Similarly, inhibition of PDE4D expression indicates that the agent is an antagonist of PDE4D activity. In another embodiment, the level and/or
25 pattern of PDE4D polypeptide(s) (e.g., different splicing variants) in the presence of the agent to be tested, is compared with a control level and/or pattern that has previously been established. A level and/or pattern in the presence of the agent that differs from the control level and/or pattern by an amount or in a manner that is statistically significant indicates that the agent alters PDE4D expression.
30 In another embodiment of the invention, agents which alter the expression of the PDE4D gene or which otherwise interact with the nucleic acids described herein,

can be identified using a cell, cell lysate, or solution containing a nucleic acid encoding the promoter region of the PDE4D gene operably linked to a reporter gene. After contact with an agent to be tested, the level of expression of the reporter gene (e.g., the level of mRNA or of protein expressed) is assessed, and is compared with
5 the level of expression in a control (i.e., the level of the expression of the reporter gene in the absence of the agent to be tested). If the level in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level in the absence of the agent, then the agent is an agent that alters the expression of PDE4D, as indicated by its ability to alter expression of a gene that is operably
10 linked to the PDE4D gene promoter. Enhancement of the expression of the reporter indicates that the agent is an agonist of PDE4D activity. Similarly, inhibition of the expression of the reporter indicates that the agent is an antagonist of PDE4D activity. In another embodiment, the level of expression of the reporter in the presence of the agent to be tested, is compared with a control level that has
15 previously been established. A level in the presence of the agent that differs from the control level by an amount or in a manner that is statistically significant indicates that the agent alters PDE4D expression.

Agents which alter the amounts of different splicing variants encoded by PDE4D (e.g., an agent which enhances activity of a first splicing variant, and which
20 inhibits activity of a second splicing variant), as well as agents which are agonists of activity of a first splicing variant and antagonists of activity of a second splicing variant, can easily be identified using these methods described above.

In other embodiments of the invention, assays can be used to assess the impact of a test agent on the activity of a polypeptide in relation to a PDE4D binding
25 agent. For example, a cell that expresses a compound that interacts with PDE4D (herein referred to as a "PDE4D binding agent", which can be a polypeptide or other molecule that interacts with PDE4D, such as a receptor) is contacted with PDE4D in the presence of a test agent, and the ability of the test agent to alter the interaction between PDE4D and the PDE4D binding agent is determined. Alternatively, a cell
30 lysate or a solution containing the PDE4D binding agent, can be used. An agent

which binds to PDE4D or the PDE4D binding agent can alter the interaction by interfering with, or enhancing the ability of PDE4D to bind to, associate with, or otherwise interact with the PDE4D binding agent. Determining the ability of the test agent to bind to PDE4D or an PDE4D binding agent can be accomplished, for example, by coupling the test agent with a radioisotope or enzymatic label such that binding of the test agent to the polypeptide can be determined by detecting the labeled with ^{125}I , ^{35}S , ^{14}C or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test agents can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. It is also within the scope of this invention to determine the ability of a test agent to interact with the polypeptide without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a test agent with PDE4D or a PDE4D binding agent without the labeling of either the test agent, PDE4D, or the PDE4D binding agent. McConnell, H.M. *et al.* (1992) *Science*, 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between ligand and polypeptide. See the Examples Section for a discussion of known PDE4D binding partners. Thus, these receptors can be used to screen for compounds that are PDE4D receptor agonists for use in treating stroke or PDE4D receptor antagonists for studying stroke. The linkage data provided herein, for the first time, provides such connection to stroke. Drugs could be designed to regulate PDE4D receptor activation which in turn can be used to regulate signaling pathways and transcription events of genes downstream, such as Cbfa1.

In another embodiment of the invention, assays can be used to identify polypeptides that interact with one or more PDE4D polypeptides, as described herein. For example, a yeast two-hybrid system such as that described by Fields and Song (Fields, S. and Song, O., *Nature* 340:245-246 (1989)) can be used to identify

polypeptides that interact with one or more PDE4D polypeptides. In such a yeast two-hybrid system, vectors are constructed based on the flexibility of a transcription factor which has two functional domains (a DNA binding domain and a transcription activation domain). If the two domains are separated but fused to two different

5 proteins that interact with one another, transcriptional activation can be achieved, and transcription of specific markers (e.g., nutritional markers such as His and Ade, or color markers such as lacZ) can be used to identify the presence of interaction and transcriptional activation. For example, in the methods of the invention, a first vector is used which includes a nucleic acid encoding a DNA binding domain and

10 also an PDE4D polypeptide, splicing variant, or fragment or derivative thereof, and a second vector is used which includes a nucleic acid encoding a transcription activation domain and also a nucleic acid encoding a polypeptide which potentially may interact with the PDE4D polypeptide, splicing variant, or fragment or derivative thereof (e.g., a PDE4D polypeptide binding agent or receptor). Incubation of yeast

15 containing the first vector and the second vector under appropriate conditions (e.g., mating conditions such as used in the Matchmaker™ system from Clontech) allows identification of colonies which express the markers of interest. These colonies can be examined to identify the polypeptide(s) which interact with the PDE4D polypeptide or fragment or derivative thereof. Such polypeptides may be useful as

20 agents which alter the activity of expression of an PDE4D polypeptide, as described above.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PDE4D, the PDE4D binding agent, or other components of the assay on a solid support, in order to facilitate

25 separation of complexed from uncomplexed forms of one or both of the polypeptides, as well as to accommodate automation of the assay. Binding of a test agent to the polypeptide, or interaction of the polypeptide with a binding agent in the presence and absence of a test agent, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test

30 tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein (e.g., a glutathione-S-transferase fusion protein) can be provided which adds a domain that

allows PDE4D or a PDE4D binding agent to be bound to a matrix or other solid support.

In another embodiment, modulators of expression of nucleic acid molecules of the invention are identified in a method wherein a cell, cell lysate, or solution
5 containing a nucleic acid encoding PDE4D is contacted with a test agent and the expression of appropriate mRNA or polypeptide (e.g., splicing variant(s)) in the cell, cell lysate, or solution, is determined. The level of expression of appropriate mRNA or polypeptide(s) in the presence of the test agent is compared to the level of expression of mRNA or polypeptide(s) in the absence of the test agent. The test
10 agent can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater (statistically significantly greater) in the presence of the test agent than in its absence, the test agent is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less
15 (statistically significantly less) in the presence of the test agent than in its absence, the test agent is identified as an inhibitor of the mRNA or polypeptide expression. The level of mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting mRNA or polypeptide.

This invention further pertains to novel agents identified by the
20 above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a test agent that is a modulating agent, an antisense nucleic acid molecule, a specific antibody, or a polypeptide-binding agent) can be used in an animal model to
25 determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein. In addition, an agent identified as described
30 herein can be used to alter activity of a polypeptide encoded by PDE4D, or to alter expression of PDE4D, by contacting the polypeptide or the gene (or contacting a cell

comprising the polypeptide or the gene) with the agent identified as described herein.

PHARMACEUTICAL COMPOSITIONS

The present invention also pertains to pharmaceutical compositions comprising nucleic acids described herein, particularly nucleotides encoding the polypeptides described herein; comprising polypeptides described herein (e.g., one or more of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14); and/or comprising other splicing variants encoded by PDE4D; and/or an agent that alters (e.g., enhances or inhibits) PDE4D gene expression or PDE4D polypeptide activity as described herein. For instance, a polypeptide, protein (e.g., an PDE4D receptor), an agent that alters PDE4D gene expression, or a PDE4D binding agent or binding partner, fragment, fusion protein or prodrug thereof, or a nucleotide or nucleic acid construct (vector) comprising a nucleotide of the present invention, or an agent that alters PDE4D polypeptide activity, can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active agents.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional

binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

5 Methods of introduction of these compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, topical, oral and intranasal. Other suitable methods of introduction can also include gene therapy (as described below), rechargeable or biodegradable devices, particle acceleration devices ("gene guns") and slow release polymeric
10 devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

 The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, compositions for intravenous administration typically are
15 solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicat-
20 ing the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

25 For topical application, nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, can be employed. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc.,
30 which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The

agent may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g.,
5 pressurized air.

Agents described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium,
10 potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The agents are administered in a therapeutically effective amount. The amount of agents which will be therapeutically effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or
15 condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the symptoms of stroke, and should be decided according to the judgment of a practitioner and each patient's
20 circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a
25 notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (e.g., separately, sequentially or concurrently), or the like. The pack
30 or kit may also include means for reminding the patient to take the therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a

plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a single vial or tablet. Agents assembled in a blister pack or other dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual

5 pharmacodynamics of each agent and administered in FDA approved dosages in standard time courses.

METHODS OF THERAPY

The present invention also pertains to methods of treatment (prophylactic and/or therapeutic) for stroke, particularly ischemic and TIA, using a PDE4D therapeutic agent. A "PDE4D therapeutic agent" is an agent that alters (e.g., enhances or inhibits) PDE4D polypeptide activity and/or PDE4D gene expression, as described herein (e.g., a PDE4D agonist or antagonist). PDE4D therapeutic agents can alter PDE4D polypeptide activity or gene expression by a variety of

15 means, such as, for example, by providing additional PDE4D polypeptide or by upregulating the transcription or translation of the PDE4D gene; by altering posttranslational processing of the PDE4D polypeptide; by altering transcription of PDE4D splicing variants; or by interfering with PDE4D polypeptide activity (e.g., by binding to a PDE4D polypeptide), or by downregulating the transcription or

20 translation of the PDE4D gene. Representative PDE4D therapeutic agents include the following:

nucleic acids or fragments or derivatives thereof described herein, particularly nucleotides encoding the polypeptides described herein and vectors comprising such nucleic acids (e.g., a gene, cDNA, and/or mRNA, such as a nucleic acid encoding a PDE4D polypeptide or active fragment or derivative thereof, or an

25 oligonucleotide; for example, SEQ ID NO: 1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10 or a nucleic acid encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14, or fragments or derivatives thereof);

polypeptides described herein (e.g., one or more of SEQ ID NO: 2, 3, 4, 5, 6,

30 7, 8, 9, 10, 12 or 14, and/or other splicing variants encoded by PDE4D, or fragments or derivatives thereof);

other polypeptides (e.g., PDE4D receptors); PDE4D binding agents; peptidomimetics; fusion proteins or prodrugs thereof; antibodies (e.g., an antibody to a mutant PDE4D polypeptide, or an antibody to a non-mutant PDE4D polypeptide, or an antibody to a particular splicing variant encoded by PDE4D, as described
5 above); ribozymes; other small molecules;

and other agents that alter (e.g., enhance or inhibit) PDE4D gene expression or polypeptide activity, or that regulate transcription of PDE4D splicing variants (e.g., agents that affect which splicing variants are expressed, or that affect the amount of each splicing variant that is expressed.

10 More than one PDE4D therapeutic agent can be used concurrently, if desired.

The PDE4D therapeutic agent that is a nucleic acid is used in the treatment of stroke. The term, "treatment" as used herein, refers not only to ameliorating symptoms associated with the disease, but also preventing or delaying the onset of the disease, and also lessening the severity or frequency of symptoms of the disease.

15 The therapy is designed to alter (e.g., inhibit or enhance), replace or supplement activity of a PDE4D polypeptide in an individual. For example, a PDE4D therapeutic agent can be administered in order to upregulate or increase the expression or availability of the PDE4D gene or of specific splicing variants of PDE4D, or, conversely, to downregulate or decrease the expression or availability of
20 the PDE4D gene or specific splicing variants of PDE4D. Upregulation or increasing expression or availability of a native PDE4D gene or of a particular splicing variant could interfere with or compensate for the expression or activity of a defective gene or another splicing variant; downregulation or decreasing expression or availability of a native PDE4D gene or of a particular splicing variant could minimize the
25 expression or activity of a defective gene or the particular splicing variant and thereby minimize the impact of the defective gene or the particular splicing variant.

The PDE4D therapeutic agent(s) are administered in a therapeutically effective amount (i.e., an amount that is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset
30 of the disease, and/or also lessening the severity or frequency of symptoms of the disease). The amount which will be therapeutically effective in the treatment of a

particular individual's disorder or condition will depend on the symptoms and severity of the disease, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also
5 depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

In one embodiment, a nucleic acid of the invention (e.g., a nucleic acid
10 encoding a PDE4D polypeptide, such as SEQ ID NO:1 which may optionally comprise at least one polymorphism shown in Tables 9 and 10; or another nucleic acid that encodes a PDE4D polypeptide or a splicing variant, derivative or fragment thereof, such as a nucleic acid encoding SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 14) can be used, either alone or in a pharmaceutical composition as described above.
15 For example, PDE4D or a cDNA encoding the PDE4D polypeptide, either by itself or included within a vector, can be introduced into cells (either *in vitro* or *in vivo*) such that the cells produce native PDE4D polypeptide. If necessary, cells that have been transformed with the gene or cDNA or a vector comprising the gene or cDNA can be introduced (or re-introduced) into an individual affected with the disease.
20 Thus, cells which, in nature, lack native PDE4D expression and activity, or have mutant PDE4D expression and activity, or have expression of a disease-associated PDE4D splicing variant, can be engineered to express PDE4D polypeptide or an active fragment of the PDE4D polypeptide (or a different variant of PDE4D polypeptide). In a preferred embodiment, nucleic acid encoding the PDE4D
25 polypeptide, or an active fragment or derivative thereof, can be introduced into an expression vector, such as a viral vector, and the vector can be introduced into appropriate cells in an animal. Other gene transfer systems, including viral and nonviral transfer systems, can be used. Alternatively, nonviral gene transfer methods, such as calcium phosphate coprecipitation, mechanical techniques (e.g.,
30 microinjection); membrane fusion-mediated transfer via liposomes; or direct DNA uptake, can also be used.

Alternatively, in another embodiment of the invention, a nucleic acid of the invention; a nucleic acid complementary to a nucleic acid of the invention; or a portion of such a nucleic acid (e.g., an oligonucleotide as described below), can be used in "antisense" therapy, in which a nucleic acid (e.g., an oligonucleotide) which
5 specifically hybridizes to the mRNA and/or genomic DNA of PDE4D is administered or generated *in situ*. The antisense nucleic acid that specifically hybridizes to the mRNA and/or DNA inhibits expression of the PDE4D polypeptide, e.g., by inhibiting translation and/or transcription. Binding of the antisense nucleic acid can be by conventional base pair complementarity, or, for example, in the case
10 of binding to DNA duplexes, through specific interaction in the major groove of the double helix.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid as described above. When the plasmid is transcribed in the cell, it produces RNA which is complementary to a portion of the
15 mRNA and/or DNA which encodes PDE4D polypeptide. Alternatively, the antisense construct can be an oligonucleotide probe which is generated *ex vivo* and introduced into cells; it then inhibits expression by hybridizing with the mRNA and/or genomic DNA of PDE4D. In one embodiment, the oligonucleotide probes are modified oligonucleotides which are resistant to endogenous nucleases, e.g.
20 exonucleases and/or endonucleases, thereby rendering them stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Pat. Nos. 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy are also described,
25 for example, by Van der Krol *et al.* ((1988) *Biotechniques* 6:958-976); and Stein *et al.* ((1988) *Cancer Res* 48:2659-2668). With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g. between the -10 and +10 regions of PDE4D sequence, are preferred.

To perform antisense therapy, oligonucleotides (mRNA, cDNA or DNA) are
30 designed that are complementary to mRNA encoding PDE4D. The antisense oligonucleotides bind to PDE4D mRNA transcripts and prevent translation.

Absolute complementarity, although preferred, is not required. a sequence "complementary" to a portion of an RNA, as referred to herein, indicates that a sequence has sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid, as described in detail above. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures.

The oligonucleotides used in antisense therapy can be DNA, RNA, or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotides can include other appended groups such as peptides (e.g. for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.*, (1987), *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT International Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT International Publication No. W089/10134), or hybridization-triggered cleavage agents (see, e.g., Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, (1988), *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent).

The antisense molecules are delivered to cells which express PDE4D *in vivo*. A number of methods can be used for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the

target cell surface) can be administered systematically. Alternatively, in a preferred embodiment, a recombinant DNA construct is utilized in which the antisense oligonucleotide is placed under the control of a strong promoter (e.g., pol III or pol II). The use of such a construct to transfect target cells in the patient results in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous PDE4D transcripts and thereby prevent translation of the PDE4D mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art and described above. For example, a plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral vectors can be used which selectively infect the desired tissue, in which case administration may be accomplished by another route (e.g., systematically).

Endogenous PDE4D expression can also be reduced by inactivating or "knocking out" PDE4D or its promoter using targeted homologous recombination (e.g., see Smithies *et al.* (1985) *Nature* 317:230-234; Thomas & Capecchi (1987) *Cell* 51:503-512; Thompson *et al.* (1989) *Cell* 5:313-321). For example, a mutant, non-functional PDE4D (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous PDE4D (either the coding regions or regulatory regions of PDE4D) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express PDE4D *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of PDE4D. The recombinant DNA constructs can be directly administered or targeted to the required site *in vivo* using appropriate vectors, as described above. Alternatively, expression of non-mutant PDE4D can be increased using a similar method: targeted homologous recombination can be used to insert a DNA construct comprising a non-mutant, functional PDE4D (e.g., a gene having SEQ ID NO:1 which may optionally comprise at least one polymorphism shown in

Tables 9 and 10), or a portion thereof, in place of a mutant PDE4D in the cell, as described above. In another embodiment, targeted homologous recombination can be used to insert a DNA construct comprising a nucleic acid that encodes a PDE4D polypeptide variant that differs from that present in the cell.

- 5 Alternatively, endogenous PDE4D expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of PDE4D (i.e., the PDE4D promoter and/or enhancers) to form triple helical structures that prevent transcription of PDE4D in target cells in the body. (See generally, Helene, C. (1991) *Anticancer Drug Des.*, 6(6):569-84; Helene, C., *et al.* (1992) *Ann. N.Y.*
- 10 *Acad. Sci.*, 660:27-36; and Maher, L. J. (1992) *Bioassays* 14(12):807-15). Likewise, the antisense constructs described herein, by antagonizing the normal biological activity of one of the PDE4D proteins, can be used in the manipulation of tissue, e.g. tissue differentiation, both *in vivo* and *for ex vivo* tissue cultures. Furthermore, the anti-sense techniques (e.g. microinjection of antisense molecules, or transfection
- 15 with plasmids whose transcripts are anti-sense with regard to a PDE4D mRNA or gene sequence) can be used to investigate role of PDE4D in developmental events, as well as the normal cellular function of PDE4D in adult tissue. Such techniques can be utilized in cell culture, but can also be used in the creation of transgenic animals.
- 20 In yet another embodiment of the invention, other PDE4D therapeutic agents as described herein can also be used in the treatment or prevention of stroke. The therapeutic agents can be delivered in a composition, as described above, or by themselves. They can be administered systemically, or can be targeted to a particular tissue. The therapeutic agents can be produced by a variety of means,
- 25 including chemical synthesis; recombinant production; *in vivo* production (e.g., a transgenic animal, such as U.S. Pat. No. 4,873,316 to Meade *et al.*), for example, and can be isolated using standard means such as those described herein.

A combination of any of the above methods of treatment (e.g., administration of non-mutant PDE4D polypeptide in conjunction with antisense therapy targeting

30 mutant PDE4D mRNA; administration of a first splicing variant encoded by PDE4D

in conjunction with antisense therapy targeting a second splicing encoded by PDE4D), can also be used.

The invention will be further described by the following non-limiting examples. The teachings of all publications cited herein are incorporated herein by
5 reference in their entirety.

EXAMPLES

EXAMPLE 1 IDENTIFICATION OF THE PDE4D GENE WITH LINKAGE TO STROKE

Icelandic Stroke Patients and Phenotype Characterization

10 A population-based list containing 2543 Icelandic stroke patients, diagnosed from 1993 through 1997, was derived from two major hospitals in Iceland and the Icelandic Heart Association (the study was approved by the Icelandic Data Protection Commission of Iceland and the National Bioethics Committee). Patients with hemorrhagic stroke represented 6% of all patients (patients with the Icelandic
15 type of hereditary cerebral hemorrhage with amyloidosis and patients with subarachnoid hemorrhage were excluded). Ischemic stroke accounted for 67% of the total patients and TIAs 27%. The distribution of stroke subtypes in this study is similar to that reported in other Caucasian populations (Mohr, J.P., *et al.*, *Neurology*, 28:754-762 (1978); L. R. Caplan, *In Stroke, A Clinical Approach*
20 (Butterworth-Heinemann, Stoneham, MA, ed 3, (1993)).

The list of approximately 2000 living patients was run through our computerized genealogy database. A comprehensive genealogy database that has been established at deCODE genetics, Inc. was used to cluster the patients in pedigrees. Each version of the computerized genealogy database is reversibly
25 encrypted by the Data Protection Commission of Iceland before arriving at the laboratory (Gulcher, J.R., *et al.*, *Eur. J. Hum. Genet.* 8:739 (2000)). The database uses a patient list, with encrypted personal identifiers, as input, and recursive algorithms to find all ancestors in the database who are related to any member on the

input list within a given number of generations back (Gulcher, J.R., and Stefansson, K., *Clin. Chem. Lab. Med.* 36:523 (1998)) covering the whole Icelandic nation. The cluster function then searches for ancestors who are common to any two or more members of the input list. One hundred and seventy-nine families with two or more
5 living patients were chosen for the study with a total of 476 patients connected within 6 meioses (6 meioses connect second cousins). Informed consent was obtained from all patients and their relatives whose DNA samples were used in the linkage scan. The mean separation between affected pairs is 4.8 meioses. Of the patients selected for the study 73% had ischemic strokes, 23% TIAs and 4%
10 hemorrhagic strokes.

In the selected families, hemorrhagic stroke patients clustered with ischemic stroke and TIA patients, and there were no families with a striking preponderance of hemorrhagic stroke or of the subtypes of ischemic stroke. Patients with ischemic stroke were reclassified according to the TOAST (Trial of Org 10172 in Acute
15 Stroke Treatment) sub-classification system for stroke (Adams, H.P., Jr., *et al.*, *Stroke*, 24:34-41 (1993)). This system includes five categories: (1) large-artery atherosclerosis, (2) cardioembolism, (3) small-artery occlusion (lacune), (4) stroke of other determined etiology and (5) stroke of undetermined etiology. The diagnoses were based on clinical features and on data from ancillary diagnostic studies.
20 Patients defined with large-artery atherosclerosis had clinical and brain imaging findings of cerebral cortical dysfunction and either significant (>70%) stenosis (this is a stricter criteria than used in TOAST where 50% stenosis is the cut-off) or occlusion of a major brain artery or branch cortical artery. Potential sources of cardiogenic embolism were excluded. The category cardioembolism included
25 patients with at least one cardiac source for an embolus and potential large-artery sources of thrombosis and embolism was eliminated. Patients with small-artery occlusion had one of the traditional clinical lacunar syndromes and no evidence of cerebral cortical dysfunction. Potential cardiac source of embolus and stenosis >70% in an ipsilateral extracranial artery was excluded. The category, acute stroke
30 of other determined etiology, included patients with rare causes of stroke and patients with two or more potential causes of stroke. If the causes of stroke could

not be determined despite extensive evaluation patients were included in the category stroke of undetermined etiology. Fig. 1A and Fig. 1B display two pedigrees each affected by several of the stroke subtypes, including hemorrhagic stroke. Apparently what is inherited in stroke is the broadly defined phenotype.

5 *Genome-wide scan*

A genome-wide scan was performed using a framework map of about 1000 microsatellite markers. The DNA samples were genotyped using approximately 1000 fluorescently labelled primers. A microsatellite screening set based in part on the ABI Linkage Marker (v2) screening set and the ABI Linkage Marker (v2) intercalating set in combination with 500 custom-made markers were developed. All markers were extensively tested for robustness, ease of scoring, and efficiency in 4X multiplex PCR reactions. In the framework marker set, the average spacing between markers was approximately 4 cM with no gaps larger than 10 cM. Marker positions were obtained from the Marshfield map (<http://research.marshfieldclinic.org/genetics>) except for a three-marker putative inversion on chromosome 8 (Jonsdottir, G.M., *et al.*, *Am. J. Hum. Genet.*, 67 (Suppl. 2):332 (2000); Yu, A., *et al.*, *Am. J. Hum. Genet.*, 67 (Suppl. 2):10 (2000). The PCR amplifications were set up, run and pooled on Perkin Elmer/Applied Biosystems 877 Integrated Catalyst Thermocyclers with a similar protocol for each marker. The reaction volume used was 5 µl and for each PCR reaction 20 ng of genomic DNA was amplified in the presence of 2 pmol of each primer, 0.25 U AMPLITAQ GOLD (DNA polymerase; trademark of Roche Molecular Systems), 0.2 mM dNTPs and 2.5 mM MgCl₂ (buffer was supplied by manufacturer). The PCR conditions used were 95°C for 10 minutes, then 37 cycles of 15 s at 94°C, 30s at 55°C and 1 min at 72°C. The PCR products were supplemented with the internal size standard and the pools were separated and detected on Applied Biosystems model 377 Sequencer using v3.0 GENESCAN (peak calling software; trademark of Applied Biosystems). Alleles were called automatically with the TRUEALLELE (computer program for alleles identification; trademark of Cybegenetics, Inc.) program (www.cybgen.com), and the program, DECODE-GT (computer editing program that works downstream

of the TRUEALLELE program; trademark of deCODE genetics, Inc.), was used to fractionate according to quality and edit the called genotypes (Palsson, B., *et al.*, *Genome Res.* 9:1002 (1999)). At least 180 Icelandic controls were genotyped to derive allelic frequencies.

5 A total of 476 patients and 438 relatives were genotyped. The data was analyzed and the statistical significance determined by applying affecteds-only allele-sharing methods (which does not specify any particular inheritance model) implemented in the ALLEGRO (computer program for multipoint linkage analysis; trademark of deCODE genetics, Inc.) program which calculates lod scores based on
10 multipoint calculations. Our baseline linkage analysis uses the S_{pairs} scoring function (Kruglyak, L., *et al.*, *Am. J. Hum. Genet.*, 58:1347 (1996)), the exponential allele-sharing model (Kong, A. and Cox, N.J., *Am. J. Hum. Genet.*, 61:1179 (1997)), and a family weighting scheme which is halfway, on the log scale, between weighting each affected pair equally and weighting each family equally. In the
15 analysis we treat all genotyped individuals who are not affected as "unknown". All linkage analyses in this paper were performed using multipoint calculation with the program ALLEGRO (deCODE genetics, Inc.) (Gudbjartsson, D.F., *et al.*, *Nat. Genet.* 25:12 (2000)).

20 The allele sharing lod scores for the genome scan using the framework map showed three regions that achieved a lod score above 1.0. Two of these regions are on chromosome 5q. The first peak is at approximately 69 cM with a lod score of 2.00. The second peak is at 99 cM with a lod score of 1.14. The third region is on chromosome 14q at 55 cM with a lod score of 1.24.

25 The information for linkage at the 5q locus was increased by genotyping an additional 45 markers over a 45 cM segment which spanned both peaks. The information used here is defined by Nicolae (D. L. Nicolae, Thesis, University of Chicago (1999)) and has been demonstrated to be asymptotically equivalent to a classical measure of the fraction of missing information (Dempster, A.P., *et al.*, *J. R. Statist. Soc. B*, 39:1 (1977)). While the lod score at the second peak dropped slightly
30 to around 1.05, the lod score at the first peak increased to 3.39. However, close inspection of our results suggested that not only does the Marshfield genetic map

(<http://research.marshfieldclinic.org/genetics>) lack resolution (many markers assigned the same map location), but also there may be some errors in their order. As a result, the genetic length of the region estimated using our material was substantially greater than what is reported. By modifying the ALLEGRO (deCODE genetics, Inc.) program, we applied the EM algorithm to our data to estimate the genetic distances between markers. We found that our estimate of the genetic length of the region was substantially longer than that given in the Marshfield map. This indicates a problem with marker order because, in general, incorrect marker order leads to an increased number of apparent crossovers and increases the apparent genetic length.

Physical and genetic mapping

The marker order and inter-marker distances were improved by constructing high density physical and genetic maps over a 20 cM region between markers D5S474 and D5S2046. A combination of data from coincident hybridizations of BAC membranes using a high density of STSs and the Fingerprinting Contig database was used to build large contigs of BACs from the RPCI -11 library. The order of the linkage markers was also confirmed by high-resolution genetic mapping using the stroke families supplemented with over 112 other large nuclear families (Fig. 3). High resolution genetic mapping was used both to anchor and place in order contigs found by physical mapping as well as to obtain accurate inter-marker distances for the correctly ordered markers. Data from 112 Icelandic nuclear families (sibships with their parents, containing from two to seven siblings) were analyzed together with the nuclear families available within the stroke pedigrees. For the purpose of genetic mapping the 112 nuclear families alone provide 588 meioses, and the total number of meioses available for mapping was over 2000. By comparison, the Marshfield genetic map was constructed based on 182 meioses. The large number of meiotic events within our families provides the ability to map markers to the resolution of 0.5 to 1.0 cM. Combining this information with the physical map resulted in a highly reliable order of markers and inter-marker distances within this 20 cM region. Linkage markers common to the genetic and

physical maps were used to anchor and place in order four of the physically mapped contigs. By integrating the genetic and physical maps a most likely order of 30 polymorphic markers was derived (Fig. 3).

BAC contigs were generated by a method that combines coincident primer
5 hybridization with data mining. The RPCI-11 human male BAC library segments 1
& 2 (Pieter de Jong, Children's Hospital Oakland Research Institute) containing
about 200,000 clones with a 12X coverage, were gridded using a 6x6 double offset
pattern in 23 cm x 23 cm membranes with a BioGrid robot (Biorobotics Ltd.,
Cambridge, UK). Initially, hybridizations were performed with markers in the
10 region of interest according to their location in the Weizmann Institute Unified
Database (<http://bioinformatics.weizmann.ac.il/udb/>). Primer sequences were
analyzed and discarded according to their content of known repeats, *E. coli* and
vector sequences (the analysis was performed using software developed at deCODE
genetics). One hundred and fifty markers in the region (30 polymorphic markers
15 used in linkage and 120 generated from STSs) separated by an average of 130 kb
were used. The selected markers were used to generate two ³²P labelled probes, F
that contained the pooled forward primers and R that contained the pooled reverse
primers. Reading of positive signals was performed automatically from digitized
images of resulting autoradiograms by informatics tools developed at deCODE
20 genetics. The coincident signals in both hybridizations were selected as positive
clones. A set of overlapping clones was assembled through a combination of
hybridization and BAC fingerprint walking. Fingerprints of positive clones were
analyzed using the FPC database developed at the Sanger Center. Data from FPC
contigs prebuilt with a cutoff of 3e-12 and from sequence datamining was integrated
25 with the hybridization results. BACs in the region detected by data mining and
hybridization were re-arrayed using a Multiprobe Ilex robot (Packard, Meriden, CT).
Small membranes (8 cm x 12 cm) were gridded in 6x6 double offset pattern and
individually hybridized with the markers of interest. Positive patterns were
transferred using transparencies to an Excel file containing macros to provide BAC
30 to marker associations. A visual map was generated by combining the hybridization,
fingerprinting and sequence data. New markers were generated from BAC end

sequences to close the gap. After several rounds of hybridization positive BACs were assembled into 7 contigs covering approximately 20 Mb. Thirty of the polymorphic markers used in linkage were assigned to four of the contigs (Fig. 3). Estimation of contig lengths and distance between markers assigned to them was
5 based on the FPC program.

Twenty - seven of our 30 linkage markers mapped to three contigs in the October 2000 release from UCSC, the UC Santa Cruz (UCSC) draft assembly (<http://genome.ucsc.edu/>). The marker order within the contigs is in agreement with our order with the exception of two markers. Although the UCSC assemblies are
10 improving, some contigs have incorrect order, orientation, or contig assembly. We believe that high resolution genetic mapping and perhaps focused hybridization experiments are still necessary to confirm accuracy of sequence assemblies. In addition, high resolution genetic mapping provides better estimates of inter-marker genetic distances that are also important for linkage analysis (Halpern, J. and
15 Whittermore, A.S., *Hum. Hered.* 49:194 (1999); Daw, E.W., *et al.*, *Genet. Epidemiol.* 19:366 (2000)).

Final linkage results and localization

Linkage analysis including genotypes from the higher density markers using the deCODE marker order resulted in a lod score of 4.40 ($P = 3.9 \times 10^{-6}$) on
20 chromosome 5q12 at the marker D5S2080. The reported P value is part of the output of the ALLEGRO (deCODE genetics, Inc.) program. It is obtained by comparing the observed lod score to the distribution of the lod score calculated under the null hypothesis of no linkage and the assumption that the descent information is complete. In this case, it agrees very well with the P value that one
25 would obtain by large sample approximation. The allele sharing lod score is the log, base 10, of an one-degree of freedom likelihood ratio. Hence, with a one-sided test, a lod score of 4.03 corresponds to a Z score of $\sqrt{2 \cdot \log(10) \cdot 4.03} = 4.31$. Normal approximation gives a P value of 8.2×10^{-6} . The locus has been designated as *STRK1*. With the addition of these extra markers, it was possible to narrow down
30 the region to a segment less than 6 cM, from D5S1474 to D5S398, as defined by one

drop in lod. Analyses using the marker orders based on publicly available marker maps gave lower lod scores, ranging from 2.78 to 3.94.

To further investigate the contribution of this susceptibility locus to stroke, a range of parametric models were fitted to the data. However, all analyses were still
5 *affecteds only* in the sense that individuals were either classified as affecteds or having unknown disease status. A lod score of 4.08 was obtained with a dominant model where the allele frequency of the susceptibility gene was assumed to be 5% and carriers of the mutation were assumed to have seven-fold the risk of a non-carrier. By inspecting the individual families, no obvious correlation was seen
10 between families which contribute positively to the linkage results with the prevalence of hypertension, diabetes or hyperlipidemias. When the data were reanalyzed with the hemorrhagic stroke patients removed, the allele sharing lod score increased to 4.86 at D5S2080. Although this 0.46 increase in log score suggests that *STRK1* is involved primarily in ischemic stroke and TIAs, it is not
15 statistically significant based on simulations (one sided P equals 0.09). In order to assess whether such a change in lod score would be likely to occur by chance we selected 1000 random sets of 22 patients whose status we then changed to "unknown" in an analysis. The P value we present is the fraction of the 1000 simulations which produce a lod score increase at the peak locus equal to or greater
20 than that which we observed by changing the affection status of the 22 hemorrhagic stroke patients to "unknown".

Identification of Allelic Association

All microsatellite markers in the approx. 6 cM interval (Fig. 3, markers from D5S398 to D5S1474) were analysed with respect to allelic association.

Table 1. The association of a fixed allele, with the stroke patients compared with population controls.

Marker	Location (cM)	Allele (A)	p-value	Risk ratio	Total no. of patients	Patients with A	Total no. of controls	Controls with A
AC022125-3	68.3	0	2.83e-03	1.28	749	412	504	251
D5S2000	68.5	0	3.26e-03	1.27	717	302	555	196
D5S2091	68.6	0	5.44e-04	1.30	757	342	534	198
D17-C	68.8	0	1.91e-03	1.34	721	436	469	249
D17-B	68.9	0	1.30e-03	1.26	680	556	509	387
AC008818-1	72.7	0	3.26e-03	1.42	739	379	619	259
D5S1990	73.9	20	3.68e-03	1.68	756	75	623	36

Comment:

The alleles have conventional values resulting after subtracting the CEPH data.

Identification of Microsatellite and SNP Haplotypes Within the Gene

Fig. 5 shows a schematic representation of the genetic map showing microsatellite and SNP haplotypes in the region of the stroke gene. Seven haplotypes are shown from the association study of Icelandic patients (804 patients).

- 5 The haplotypes indicated as SW-1 and SW-2 are from an association study on Swedish stroke patients.

- 10 A total number of 804 Icelandic patients were analyzed for microsatellite single marker and multimarker association. The number of controls used in the analysis was 504. Each patient had 2 or more close relatives genotyped in order to derive haplotypes. The haplotypes were derived using ALLEGRO based haplotype analysis (results shown in Table 2).

Table 2
Icelandic Patient Association

Markers	Alleles	pAllelic	All Frq Aff	All Frq Ctrl	pCarrier	Carr Frq Aff	Carr Frq Ctrl	# aff	# ctrl
All patients (n=804)									
D5S2000	0	1.12E-04	0.24	0.18	5.36E-04	0.43	0.33	744	429
D5S2091	0	5.28E-04	0.26	0.21	6.10E-04	0.46	0.37	770	478
AC022125-3	0	5.96E-04	0.33	0.27	3.24E-04	0.55	0.45	774	489
D17-C	0	9.93E-04	0.36	0.29	0.007	0.6	0.52	756	395
AC008833-6	0	0.0013	0.67	0.61	0.018	0.88	0.84	781	472
AC008818-1	0	0.0014	0.29	0.24	7.13E-04	0.51	0.41	773	482
AC008829-5	2	0.0063	0.03	0.015	0.005	0.06	0.03	645	474
(1) D5S2000 D5S2091 D17-C D17-B	0000	0.0018	0.17	0.11	0.004	0.3	0.22	552	325
(2) D5S2091 D17-C D17-B	000	9.06E-04	0.19	0.13	0.001	0.34	0.25	597	380
(3) AC008829-5 AC008833-2 AC008833-3	20 14 6	0.0017	0.01	0.002	0.002	0.029	0.004	579	431
(4) AC022125-3 AC008833-6 D5S2000 D5S2091 D17-C	00000	0.00374	0.17	0.13	0.012	0.32	0.24	629	317
(5) D5S2071 AC008879-2 AC008818-1 AC008879-3	-2 0 0 0	0.0031	0.05	0.02	0.004	0.09	0.044	489	362
(6) AC008879-2 AC008818-1 AC008879-3	0 0 0	9.25E-04	0.29	0.23	5.82E-04	0.5	0.4	621	443
(part 7) D5S2107 AC008829-5 AC008833-2	4 2 0	0.0097	0.007	0	0.009	0.01	0	540	422

Swedish patients have also been genotyped and microsatellite single and multimarker association has been analyzed using the E-M algorithm. A total number of 943 Swedish patients (stroke patients and patients with carotid stenosis) and 322 Swedish controls were analyzed (results shown in Table 3).

Table 3
Swedish Patient Association

Markers	Alleles	pAllelic	All Frq Aff	All Frq Ctrl	# aff	# ctrl
Swedish patients (n=943)						
D5S2000	2	2.39E-03			912	318
(Sw 2) AC022125-3 AC008833-6 D5S2000 D5S2091	0 0 2 0	6.0E-03	0.035	0.014	717	284
(Sw-1) AC008804-2 D17-H D17-G D5S2080	-2 4 -2 10	2.8E-03	0.057	0.053	672	113
AC008804-2 D17-H D17-G	-4 0 -2	3.7E-03	0.056	0.033	700	123

SNP haplotypes within the PDE4D gene have been identified. A total of 95 SNP's typed for approximately 500 patients and 140 controls and E-M algorithm was used to analyze the genotype (results shown in Table 4). Selected SNP's found in excess in patients (based on the E-M algorithm) were typed for a subset of
5 relatives in order to derive haplotypes for haplotype analysis (results are shown in Table 5). SNP haplotypes 1 and 2 are located upstream of D6 exon, SNP haplotype 3 is located upstream of D8 exon and stretches over it, SNP haplotype 4 stretches over LF1 exon.

Table 4
SNP genotype analysis based E-M algorithm

SNP haplotype	Position	Alleles in Haploypye	pAllelic	All Frq Aff	All Frq Ctrl	#Aff	#Ctrl
SNP-1	1273143- 1269965	122303	9.9E-03	0.32	0.25	505	155
SNP-2	1260358- 1254849	10323	2.8E-02	0.33	0.26	631	131
SNP-3	1399767- 1318510	2313002	8.9E-03	0.26	0.18	759	149
SNP-4	1422008- 1410824	111330	3E-02	0.56	0.48	344	128

Table 5A
SNP haplotype analysis

SNP haplo- type	Position	Alleles in haplo- type	pAllelic	All Frq Aff	All Frq Ctrl	Carr Frq Aff	Carr Frq Ctrl	# Aff	# Ctrl
SNP-1	1273143- 1269965	122303	4.27E-04	0.31	0.18	0.49	0.308	111	149
SNP-2	1260358- 1254849	10323	0.0043	0.32	0.2	0.508	0.35	114	128

Table 5B
SNPs in the identified SNP haplotypes

Haplotype	SNP	Public name if available	Polymorphism	position	Allele
SNP-2	1	new	T/C	1254849	3
SNP-2	2	new	A/G	1257206	2
SNP-2	3	TSC0538885	T/C	1257624	3
SNP-2	4	new	A/C	1259581	0
SNP-2	5	rs244579	T/C	1260358	1
SNP1	1	rs35284	T/C	1269965	3
SNP1	2	rs35283	A/G	1270041	0
SNP1	3	rs35281	A/G	1270553	3
SNP1	4	rs35280	G/A	1272125	2
SNP1	5	new	A/G	1272910	2
SNP1	6	rs35279	G/C	1273143	1
SNP3	1	rs255652	A/G	1318510	2
SNP3	2	rs27547	G/A	1371388	0
SNP3	3	rs26695	G/A	1390407	0
SNP3	4	rs27773	C/T	1391020	3
SNP3	5	rs1471430	C/G	1391818	1
SNP3	6	rs26705	C/T	1392198	3
SNP3	7	rs26701	G/C	1399767	2
SNP4	1	rs464311	A/G	1410824	0
SNP4	2	rs1867725	T/C	1412604	3
SNP4	3	rs153966	T/C	1414091	3
SNP4	4	new	C/T	1414804	1

Table 6A and 6B show previously known microsatellite markers and novel microsatellites in sequence. Forward and reverse primers are shown.

Table 6A Previously Known microsatellite markers in sequence

	Accession number	Forward primer	SEQ ID NO.	Reverse primer	SEQ ID NO.
D5S2107	GDB:614475	AGCCTTTGGGCCAACA	15	CAAAACCAACAGGAGTATGTACTTTT	16
D5S468	GDB:593646	AAATGAATGGTAGATTAAACCTGAG	17	TGGGAAAATAAATACATGCG	18
D5S2000	GDB:608769	TTATACCAGGAGAGTAGACTTTTTT	19	CATGCTAAATTTCAAAATATGAGAG	20
D5S2091	GDB:613806	GCAATTGTTCATGTGCCA	21	GGTATTTTCATTACAGCCAGTC	22
D5S2500	GDB:683034	TTAAAGGAGTGATCTCCCCC	23	GTTACAGTACCTATGGTCATGCC	24
D5S2080	GDB:613188	GCACTGTGAATTTCAAATG	25	GTCAGGGGACTGGGAT	26
D5S2018	GDB:609957	CCTGTAAACAATGAAAACCCACTGA	27	AGACTATGCTGTGTGTGTCCTG	28
D5S2071	GDB:612756	TCTGGGTTTACAAACCTTCAAA	29	TAACTGGCTTGGCCCG	30

Table 6B Novel microsatellites in sequence:

	Forward primer	SEQ ID NO.	Reverse primer	SEQ ID NO.
DG5S382	CAGTAAATAGTTTGCTTCAGGCATT	31	CTCATACTCTGCGTGGCTTG	32
AC008829-5	AGGGCTAAGTGGATCACAGC	33	AGAGGGTCTTGCCACCTGTG	34
AC008833-2	TCTGCAAGACTCTCGGTGCT	35	TGCAGATCTCATATTTCCATGTTT	36
AC008833-3	TCTGCCCTTTGTTCCCTCATC	37	GTCAAAGGGAGTGATGGCAGT	38
AC022125-3	AAAATGACTGCCCTCCACAA	39	GGGAAATCATACTGCCCTCA	40
AC008833-6	AAACATAGCCACCCCTGTTC	41	TCCAAAGCCCTTAGCTTAATCA	42
D17-C	GCTCCCTGGACTGTGTAA	43	GCCACATTTGCTGTCAATTT	44
D17-B	TTTTTCAGGGCTGGGTAGAA	45	TCCAAAGGAAGTGAAATCAGTG	46
D17-D	CTAACCCATCCTCACCCCAAT	47	TGTGGCATACAGGGAAGTGA	48
AC008804-1	GTGCTGGAAATTTGGCTCCTA	49	CAAAACATCATTTTGCCCTTGC	50
AC008804-2	TCCCAAAACGATAGCTGTTC	51	GAATTAGGAGCGTGGCTCAA	52
AC008804-3	TTTGCAATTCATCACTCATTCG	53	CCCGTAGCATCTGATCCAGT	54
D17-H	AGAAAGCTTCCCTCCACTG	55	CATTCCAGCCTGAGCTACAA	56
D17-G	TGGGCTCCAATTATCCTTCC	57	TGCAGTTTGCACCTCCTTG	58
AC027322-12	TTATCTGTTCCTCCATGCTTTT	59	TGTTACATCTTGATCTATGACGTTT	60
AC027322-10	TGTATCCTGCAATCCCTTGTT	61	GGAAATAACCCAAAAGTAATTGTAGTGA	62
AC027322-9	TCGTGCCAAGATGAAATGA	63	AAACCTCCCTGATCATCTGAA	64
AC027322-8	ACAGAGGAGCAAAGGAATCA	65	TTGGCACGAATCACTCTCTG	66
AC027322-3	CCCAATTTGGATGATGGTAA	67	TGAGAACATCTAACGCTTTTTCAA	68
AC027322-5	GGCACAGATAACTGGGAAGC	69	CCCCCAAAGTACTGCATAAA	70
DG5S397	ATGTTGGCATTTTGGTGAGGT	71	CACCTGTCCCTTGGAGGTA	72
AC008879-2	TTTTAAACGTGAAAGTACAAAGTTGC	73	ACAAAGAGCACCTTCCAGTG	74
AC008818-1	TGCTTGGTGAAAGGAATAGCC	75	GAGCCTGGTTCTCAGGAAT	76
AC008879-3	GGCAAGAACAGTTTGGAGGA	77	GACTGCTGTTGCTGGTTGA	78
AC020733-1	AAATGGCTATAAAGTCTTTGAAC	79	CGGTCTCAACAACCCAGAAC	80
AC016591-2	CAGAAACACACAGAAAGTCAATCAA	81	CAGACCCCAATTAATGGCAAAA	82
DG5S405	TCTGTCTTCTTTGACCCCATGAAT	83	CAACACAGCGAGACCTCATC	84

Discussion of Stroke Locus Identification

Genealogy, a comprehensive population based list of broadly defined stroke patients and non-parametric allele sharing methods have been combined to successfully map a major gene for one of the most complex diseases known. There was no correlation between the contribution of the families to the locus and hypertension, diabetes or hyperlipidemias and this locus does not match any known gene contributing to these risk factors. The types of stroke studied in this work do not reflect a rare or Icelandic-specific form of stroke; rather, the diversity of the stroke phenotypes in Icelanders as well as risk factors are similar to those of most other Caucasian populations (Agnarsson, U., *et al.*, *Ann. Intern. Med.*, 130:987 (1999); Eliasson, J.H., *et al.*, *Læknablaðið*, 85:517-25 (1999); Sveinbjörnsdóttir, S., *et al.*, Systematic registration of patients with Stroke and TIA admitted to The National University Hospital, Reykjavik, Iceland, in 1997, XIII. Meeting of the Icelandic Association in Internal Medicine, Akureyri, Iceland (*Læknabladid*, 1998); Valdimarsson, E.M., *et al.*, *Læknabladid* 84:921 (1998)).

The known genetic factors contributing to common stroke may do so indirectly by increasing the risk of some of its risk factors such as diabetes, hyperlipidemias, and hypertension. It is possible that there are genetic factors for stroke that do not influence susceptibility to the known risk factors, as has been suggested by epidemiologic studies for myocardial infarction (Friedlander, Y., *et al.*, *Br. Heart J.*, 53:382 (1985); Shea, S., *et al.*, *J. Am. Coll. Cardiol.*, 4:793 (1984); Myers, R.H., *et al.*, *Am. Heart J.*, 120:963 (1990)). Epidemiological studies of the common forms of stroke have given conflicting results regarding the role of family history. Some studies have shown that parental history predicts the risk of stroke independently from conventional risk factors (Liao, D., *et al.*, *Stroke*, 28:1908 (1997); Jousilahti, P., *et al.*, *Stroke*, 28:1361 (1997)) whereas others have failed to find evidence for such independent factors (Graffagnino, C., *Stroke*, 25:1599 (1994); Kiely, D.K., *et al.*, *Stroke*, 24:1366 (1993); Lindenstrom, E., *et al.*, *Neuroepidemiology*, 12:37 (1993)).

The work described herein is the first reported genome scan searching for genes that contribute to stroke as defined as a public health problem. The data reported herein suggests that the mapped gene contributes directly to stroke without contributing indirectly through its known risk factors. This suggests that there may be other
5 biological pathways contributing to the pathogenesis of stroke.

EXAMPLE 2 IDENTIFICATION OF THE PDE4D GENE

Sequence of the Candidate Region

We have sequenced approximately 3 Mb of the area defined by one drop in lod (Fig. 3, the genetic map of the region). The BAC (bacterial artificial clones) sequenced
10 in house are shown in Table 7A. We also used for the assembly the following publicly available BAC sequences from GenBank listed in Table 7B for the assembly. The BAC clones we sequenced are from the RCPI-11 Human BAC library (Pieter deJong, Roswell Park). The vector used was pBACe3.6. The clones were picked into a 96 well microtiter plate containing LB/chloramphenicol (25 µg/ml)/glycerol (7.5%) and stored
15 at -80°C after a single colony has been positively identified through sequencing. The clones can then be streaked out on a LB agar plate with the appropriate antibiotic, chloramphenicol (25 µg/ml)/sucrose (5%).

Table 7A

Sequenced at Decode

(BAC name)	Comment	Accession number
RP11-621C19	1	AC020733
RP11-113C1	2	
RP11-412M9	2	
RP11-151G2	2	
RP11-151F7	2	
RP11-281M3	2	
RP11-421L6	2	
RP11-68E13	2	
RP11-379P8	2	
RP11-1A7	1	AC008111
RP11-422K3	2	
RP11-116A3	2	

Key to "Comment" column:

1= This BAC has a publicly available sequence,

it was sequenced at Decode to make sure the sequence was correct

2= Only BAC end-sequence available for this BAC publicly.

Table 7B

Sequences available from

GenBank (BAC name)	Accession number	Status of sequence
RP11-621C19	AC020733	17 unordered pieces
CTD-2003D5	AC016591	complete sequence
CTD-2210C1	AC008879	7 unordered pieces
CTD-2124H11	AC008818	complete sequence
CTD-2301A11	AC008934	complete sequence
RP11-16B11	AC011929	7 unordered pieces
CTC-261E10	AC026693	complete sequence
CTD-2027G10	AC027322	complete sequence
RP11-1A7	AC008111	8 unordered pieces
CTD-2122K7	AC012315	complete sequence
CTD-2085F10	AC008804	complete sequence
CTD-2040J22	AC008791	complete sequence
RP11-235N16	AC020975	16 ordered pieces
CTD-2146O16	AC008833	complete sequence
CTD-2084I4	AC022125	17 ordered pieces
CTD-2140K22	AC008829	26 ordered pieces
CTD-2124D11	AC020924	7 ordered pieces
RP11-731H6	AC026095	21 unordered pieces

Gene identification

The gene, human cAMP specific phosphodiesterase 4D (HPDE4D) was identified in the sequenced region (Fig. 3). Twenty-three exons have been identified,

eighteen of those have previously been published. See top of Fig. 4. Five new spliced exons have been identified (referred to as 4D6, 4D7-1, 4D7-2, 4D7-3 and 4D8) in three new isoforms (PDE4D6, PDE4D7 and PDE4D8). The genomic sequence is approximately 1,691,140 bases in length.

The exon locations are indicated in Table 8 below.

Table 8

Exon	Start	End
(New) 4D7-1	142207	142328
(New) 4D7-2	444645	444775
(New) 4D7-3	641649	641878
4D4	736254	737226
4D5	861791	862202
4D3	1044051	1044190
(New) 4D6	1273404	1273709
(New) 4D8	1354347	1355128
LF1	1414511	1414702
LF2	1436943	1436979
LF3	1472965	1473235
LF4	1449835	1449542
N3	1539259	1539302
4D1/D2	1591172	1591425
ex3	1636944	1637037
ex4	1638406	1638578
ex5	1639508	1639606
ex6	1640491	1640655
ex7	1641818	1641917
ex8	1653070	1653224
ex9	1653943	1654065
ex10	1654576	1654758
ex11	1655335	1655747

The markers showing the highest association are located within the PDE4D (Table 1, Fig. 3 and Table 5), as follows:

AC022125-3, 21 000 bp upstream of the LF1 exon
D5S2000, 37 000 bp downstream of PDE4D6 exon
D5S2091, 30 000 bp downstream of PDE4D6 exon
D17-C, 21 000 bp upstream of PDE4D6 exon
D17-B, 31 000 bp upstream of PDE4D6 exon
AC008833-6, 35 000 bp downstream of PDE4D8 exon
AC008818-1, 3000 pb upstream of PDE4D7-1 exon
AC008829-5, 89 000 bp upstream of PDE4D1/D2 exon
Haplotype (1) and (2) are located upstream of and stretch over the PDE4D6 exon
Haplotype (3) is located upstream of and stretches over the LF2-LF4 exons
Haplotype (4) stretches over PDE4D6 and PDE4D8 exons
Haplotype (5) stretches over PDE4D7-1 to PDE4D7-3 exons
Haplotype (6) stretches over PDE4D7-1 exon
Haplotype (7) stretches over LF2-exons 11

A contig for the incomplete genomic sequence of the PDE4D gene was submitted in November 2000 (GenBank entry NT_023193 by International Human Genome Project collaborators). The size of the contig is 614 481 bp (including gaps) whereas our genomic sequence for the whole PDE4D region (i.e., from the first exon for PDE4D variant) is close to 1,700,000 bp. The contig NT_023193 comprises only 11 exons of the PDE4D gene (in Fig. 4, exons 4D1/D2 - 11) and the 5' differently spliced exons are missing in the contig (in Fig. 4, exons 4D4, 4D5, 4D3, 4D6, 4D8, 4D7-1, 4D7-2, 4D7-3, LF1, LF2, LF3 and LF4).

SNPs (single nucleotide polymorphisms) detected in the sequence and mutation analysis

Publically available and novel SNP's in the PDE4D2 gene from mutation screening of all exons are illustrated in Tables 9 and 10.

Gene Identification

The identified gene PDE4D is a member of the cyclic nucleotide phosphodiesterases (PDEs). Intracellular levels of cyclic AMP and cyclic GMP are mediated by the PDEs. Cyclic nucleotides are important second messengers that regulate and mediate a number of cellular responses to extracellular signals, such as hormones, light and neurotransmitters. Intracellular levels of cAMP play a key role in the function of inflammatory and immune cells. One of the mechanisms that mediate relaxation of vascular muscle in cerebral circulation is the production of cAMP.

PDE4D Structure and Splice Forms

Phosphodiesterases are the mammalian homolog of the "dunce" gene in *Drosophila melanogaster*, implicated in learning and memory (Davis, R.L. and B. Dauwalder, *Trends Genet.*, 7(7):224-229 (1991)). PDEs are members of a large superfamily of isoenzymes subdivided into 9 and possibly 10 distinct families (Conti, M. and S.L. Jin, *Prog. Nucleic Acid Res. Mol. Biol.*, 63:1-38 (1999)), with several genes in each family and more than one isoform for each gene. The significance of the diversity of PDEs is not known but many of the isoforms differ in their biochemical properties, phosphorylation, intracellular targeting, protein-protein interactions and patterns of expression in tissues, which suggests that each of the various isoforms might have distinct functions (Bolger, G.B., *Cell Signal*, 6(8):851-859 (1994); Conti, M., *et al.*, *Endocr. Rev.*, 16(3):370-378 (1995)).

There are four genes that encode the type 5 PDEs (PDE4A, PDE4B, PDE4C and PDE4D), which is a group of enzymes characterized by high affinity for cAMP. The gene for PDE4D was assigned to human chromosome 5q12 (Milatovich, A., *et al.*, *Somat. Cell Mol. Genet.*, 20(2):75-86 (1994); Szpirer, C., *et al.*, *Cytogenet. Cell Genet.*, 69(1-2):22-14 (1995)) and 5 distinct splice variants have been characterized (the short forms PDE4D1, PDE4D2 and the long forms PDE4D3, PDE4D4, and PDE4D5) (Bolger, G.B., *et al.*, *Biochem. J.*, 328(Pt.2):539-548 (1997)) (Fig. 4). The sequence of

the human PDE4D variants show a high degree of homology to the PDE4Ds expressed in mouse and rat. The pattern of splicing and different promoter usage is highly conserved during evolution indicating an important physiological role (Nemoz, G., et al., *FEBS Lett.*, 384(1):97-102 (1996)). The PDE4D variants are generated at two major boundaries present in the gene. The first boundary corresponds to the junction of exon 2. Differential splicing in this region generates the 2 short variants PDE4D1 (586 a.a.) and PDE4D2 (508 a.a.)(Fig. 4). This splicing boundary is conserved in mouse, rat and between different human PDE4 genes. The splicing variant PDE4D2 is generated by the removal of 256 bp from the PDE4D1 sequence. The initiation codon in the PDE4D2 variant lies within exon D1/D2. Data demonstrates that the expression of the short PDE4D variants is under the control of an internal promoter regulated by cAMP (Vicini, E. and M. Conti, *Mol. Endocrinol.*, 11(7):839-850 (1997)). The second major splicing boundary is also conserved during evolution and is identical to that described in the *Drosophila dunce* gene. Splicing occurs at the intron/exon boundary at the LF1 exon (Fig. 4).

PDE function

The PDEs serve at least four major functions in the cell. They can (1) act as effector of signal transduction by interacting with receptors and G-proteins; (2) integrate the cyclic nucleotide-dependent pathway with other signal transduction pathways; (3) function as homeostatic regulators, playing a role in feedback mechanisms controlling cyclic nucleotide levels during hormone and neurotransmitter stimulation; (4) play an important role in controlling the diffusion of cyclic nucleotides and in creating subcellular domains or channeling cyclic nucleotide signaling (Conti, M. and S.L. Jin, *Prog. Nucleic Acid Res. Mol Biol.*, 63:1-38.(1999)). Inhibition of PDE has long been recognized as an effective pharmacological strategy to alter intracellular cyclic nucleotide levels (Flamm, E.S., et al., *Arch. Neurol.*, 32(8):569-71 (1975)).

It has been reported that PDE4 is the predominant isozyme regulating vascular tone mediated by cAMP hydrolysis in cerebral vessels (Willette, R.N., *et al.*, *J. Cereb. Blood Flow Metab.*, 17(2):210-9 (1997)).

A recent study on mice with targeted disruption of PDE4D gene (Hansen, G., *et al.*, *Proc. Natl. Acad. Sci. U S A*, 97(12):6751-6 (2000)) has demonstrated a crucial role of PDE4D in the control of smooth muscle contraction and muscarinic cholinergic receptor signaling but not in the control of airway inflammation. The lung phenotype of the PDE4D^{-/-} mice demonstrates that this gene plays a nonredundant role in cAMP homeostasis. There is a significant reduction in PDE activity and an increase in resting and stimulated cAMP levels in the lung, indicating that other PDE4s (or other PDEs) are not up-regulated and cannot compensate for the loss of PDE4D. These findings support that PDE4D serves a unique, nonoverlapping functions in cell signalling.

No clear link between an established inherited disorder and known PDE loci has emerged, with the exception of PDE6. Inhibitors of PDEs have been shown to affect airway responsiveness and pulmonary allergic inflammation (Schudt, C., *et al.*, *Pulm. Pharmacol. Ther.*, 12(2):123-9 (1999)). There are reports suggesting that altered PDE4 function may be linked to nephrogenic diabetes insipidus (Takeda, S., *et al.*, *Endocrinology*, 129(1):287-94 (1991)) or atopic dermatitis (Chan, S.C., *et al.*, *J. Allergy Clin. Immunol.*, 91(6):1179-88 (1993)), however no mutations have been identified. It has also been reported that that vasorelaxation modulated by PDE4 (not mentioned whether it is A, B, C or D gene family) is compromised in chronic cerebral vasospasm associated with subarachnoid hemorrhage (Willette, R.N., *et al.*, *J. Cereb. Blood Flow Metab.*, 17(2):210-9 (1997)). PDE4D itself has not been linked to stroke before.

PDE4D expression and cellular localization

PDE4Ds are expressed in human peripheral mononuclear cells (Nemoz, G., *et al.*, *FEBS Lett.*, 384(1):97-102 (1996)), brain (Bolger, G., *et al.*, *Mol. Cell Biol.*, 13(10):6558-71 (1993)), heart (Kostic, M.M., *et al.*, *J. Mol. Cell Cardiol.*,

29(11):3135-46 (1997)) and vascular smooth muscle cells (Liu, H. and D.H. Maurice, *J. Biol. Chem.*, 274(15):10557-65 (1999)).

Immunoblotting of rat brain has shown that the PDE4D3, PDE4D4 and PDE4D5 proteins are present in brain (Bolger, G.B., *et al.*, *Biochem. J.*, 328(Pt 2):539-48 (1997)) and are expressed in cortex and cerebellum from rat (Iona, S., *et al.*, *Mol. Pharmacol.*, 53(1):23-32 (1998)). These proteins were recovered mostly or exclusively in the particulate fraction suggesting that these forms may be targeted to insoluble cellular structures. In addition a 68 kDa protein was detected which could represent PDE4D1, PDE4D2 or both. To verify this RT-PCR was performed on mRNA from rat brain and the results showed that transcripts for PDE4D1 and 2 were present. Their data also suggests that the N-terminal regions of the PDE4D3-5, derived from alternatively spliced regions of their mRNAs, are important in determining their subcellular localization activity and differential sensitivity to inhibitors and there are indications that there is a propensity for the long PDE4D isoforms to interact with particulate fraction of the cell.

Newly identified isoforms

Five new exons have been identified. Exon D6 was identified by deCODE (in silico) and verified by RT-PCR. The four other new exons have been identified using CAP-RACE amplification from cultured cells with an "long-form 1"-specific reverse primer. Three of these exons are spliced to one another and together onto LF1 and this new isoform was given the name D7. The fourth new 5' exon was spliced by itself onto LF1 and given the name D8. These constitute two previously unknown isoforms.

In terms of genomic structure, the D7 exons extend the known 5' end of PDE4D over 590,000 bp and the D8 exon lies between two previously recognized exons. The D7 isoform has an open reading frame extending into LF1, resulting in an additional 90 amino acids at the N-terminus of the predicted protein. The D8 5' exon contains a long 5' UTR, followed by an ATG near the end of the exon that extends an ORF into LF1 and results in a novel 21 N-terminal amino acids in the predicted protein.

Table 11: New Isoforms

Isoform				
Name		Cell line		
	Exon	Size		
PDE4D6	D6			
PDE4D7	D7-1	5'	122 bp	SKNAS
PDE4D7	D7-2	Internal	131bp	SKNAS
PDE4D7	D7-3	Internal	230 bp	SKNAS
PDE4D8	D8	5'	782 bp	HeLa

The sequences are as follows:

D7-1:

ATAGTTGGCGTACCCTGAGGCCTGCCAGTTCCTGCCTTAATGCATATGTAGT
CGTAATTGAGTTCTGACACGGCCTTGGATGTTTCTGTCCTAAATAGCTGACA
TTGCATCTTCAAGACTGT

D7-2:

CATTCCAGTTGGCTTTTGAGTGGATACGTGCAGTGAGATCATTGACACTGGA
AACACTAGTTCCCATTTTAATTACTTAAAACACCACGATGAAAAGAAATACC
TGTGATTTGCTTTCTCGGAGCAAAAAGT

D7-3:

GCCTCTGAGGAAACACTACATTCCAGTAATGAAGAGGAAGACCCTTTCCGC
GGAATGGAACCCTATCTTGTCCGGAGACTTTCATGTCGCAATATTCAGCTTC
CCCCTCTCGCCTTCAGACAGTTGGAACAAGCTGACTTGAAAAGTGAATCAGA
GAACATTCAACGACCAACCAGCCTCCCCCTGAAGATTCTGCCGCTGATTGCT
ATCACTTCTGCAGAATCCAGTGG (SEQ. ID NO.: 11; includes D7-1, D7-2 and D7-3)

New predicted amino-terminal protein sequence from above (PDE4D7):

MKRNTCDLLSRKSASEETLHSSNEEEDPFRGMEPYLVRRLSCRNIQLPPLAFRQ
LEQADLKSESENIQRPTSLPLKILPLIAITSAESS (90 amino acids) (SEQ ID NO.:12)

D8:

TTCTCACTGCCCTGCGGTGTTTTGAACTGCCTTCTTACAGACGTCATACAGCC
CTTGAGGAATAGTTTCTGCCTGGTGAGATTGAATGATAGTTCTCATTACAA
AACCCTGGATTCTAAGCAGGGACACACAGAAATTACTTTTCGCAGGTAAATC
AGCCCACCCAGCCAAAGTGTGGAGAGATTTGTTCCCTTGGCTGACTTCTTTGC
TCCACGGAGAGGAGTGTTTTCTGTGCTTGCCCTGAAATGGAACCTCCTTGA
CAGCTCTCCCGTGTTACAGTACCTCCCGGTCATTTTCTTTTTCTCTCTCTAC
CTGCGCTCTTCGAGTGTGAGAAACCTTTAAAGCTGTTACTATGGAATTGCAA
AAAAGAGATCAAGTGACTCTTTCCTATGCTGGTTTCCCTTGTGACCCAGAT
GAAGAATCAATTCAGAATTCAGTTCCTCCCTTGGCATTGCAAGACACAGAAG
AAACTGTCACTTCCTAACAGCCTAGTACTGGAGTAAATTCAGTATGAAGGAA
GAAAGCGCTCCTGCGTGTTAGAACCTTGCCCATGAGCTGGACCGAGGACAG
GAGATGGACTCCAGGAAAATTGGATTTCTTCAAGCAGCCTCCCTTGGAAATG
GAATATCTTTAAAATCTTCTTTGCAGAAAGACAGTTAGAATGTATTAATCAG
AATAGTTGAAGACTTATTTTCCTTTTATTTTTTTTCAAATGAGCATTATTAT
GAAGCCAAGATCCCGATCTACAAGTTCCTAAGGACTGCAGAGGCAGTTTG
(SEQ ID NO.:13)

New predicted amino-terminal protein sequence from above (PDE4D8):

MSIIMKPRSRSTSSLRTAEAV (21 amino acids) (SEQ ID NO.: 14).

Expression analysis

The tissues below were examined by RT-PCR, cloning and sequencing. The presence (Pos.) or absence (-) of the isoforms transcripts is shown in tables below.

Table 12A Original Cell Lines (SKNAS and HeLa)

	D7	D8
HeLa	-	Pos.
SkNAs	Pos.	Pos.

Table 12B Human tissue DNA panels

cDNA panels	D7	D8
Spleen	-	Pos.
Lymph node	Pos.	Pos.
Thymus	Pos.	Pos.
Tonsil	Pos.	Pos.
Leukocytes	Pos.	Pos.
Bone marrow	Pos.	Pos.
Heart	-	Pos.
Brain	-	Pos.
Placenta	Pos.	Pos.
Lung	Pos.	Pos.
Liver	-	Pos.
Skel. muscle	-	Pos.
Kidney	Pos.	Pos.
Pancreas	-	Pos.

Table 12C Human blood cell fractions

	D7	D8
Spleen	Pos.	Pos.
Lymph node	Pos.	Pos.
Thymus	Pos.	Pos.
Tonsil	Pos.	Pos.
Leukocytes	Pos.	-
Bone marrow	Pos.	Pos.
Fetal liver	Pos.	Pos.
Mononucl. cells resting	Pos.	Pos.
CD4Pos. resting	-	Pos.
CD8Pos. resting	-	-
CD14Pos. resting	Pos.	Pos.
CD19Pos. resting	Pos.	Pos.
Mononucl. cells activated	-	-
CD4Pos. activated	-	-
CD8Pos. activated	-	-
CD19Pos. activated	-	Pos.

Table 12D Cultured in-house endothelial and smooth muscle cells from patients

Cell type	D1	D2	D3	D5	D6	D7	D8
Normal aorta smooth musc.	Pos.	Pos.	Pos.	Pos.	Pos.	-	-
Diseased aorta smooth musc.	Pos.	Pos.	-	Pos.	Pos.	-	Pos.
Diseased aorta smooth musc.	Pos.	Pos.	-	Pos.	Pos.	-	-
Diseased femoral smooth musc.	Pos.	Pos.	-	Pos.	Pos.	-	Pos.
Normal aortic endothelial cells	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Diseased aortic endothelial cells	Pos.	Pos.	-	Pos.	Pos.	-	-
Diseased femoral endothelial cells	Pos.	Pos.	-	Pos.	Pos.	-/?	-/?

Isoform specific primers were designed in order to better determine the expression of different PDE4D isoforms using RT-PCR on Epstein Barr Virus (EBV) transformed B cell lines from stroke patients and controls. The results are outlined in Tables 13A and 13B below. There is a significant difference between the expression of D3 and D7 in patients compared to controls.

Table 13A RT-PCR on EBV transformed B stroke patient cells

Patient	PDE4D*	D3	D4	D5	D6	D7	D8
Cells							
P-1	Pos.	Pos.	-	Pos.	-	Pos.	Pos.
P-2	Pos.	Pos.	-	Pos.	-	Pos.	-
P-3	Pos.	-	-	Pos.	-	-	-
P-4	Pos.	Pos.	-	Pos.	-	Pos.	-
P-5	Pos.	Pos.	Pos.	Pos.	-	Pos.	-
P-6	Pos.	-	Pos.	Pos.	-	Pos.	-
P-7	Pos.	Pos.	-	Pos.	-	Pos.	-
P-8	Pos.	-	-	-	-	Pos.	-
P-9	Pos.	-	-	Pos.	-	Pos.	-
P-10	Pos.	-	-	Pos.	Pos.	Pos.	-
P-11	Pos.	-	-	Pos.	-	Pos.	-
P-12	Pos.	-	-	Pos.	-	Pos.	-
P-13	Pos.	-	-	Pos.	-	Pos.	-
P-14	Pos.	-	-	Pos.	-	Pos.	-
% expr.	100	35,7	14,3	92,8	7,1	92,8	7,1

*Primers designed for the common region of PDE4D identical for all isoforms

Table 13B RT-PCR on EBV transformed B control cells

Control	PDE4D	D3	D4	D5	D6	D7	D8
Cells	*						
C-1	Pos.	-	-	Pos.	-	-	Pos.
C-2	Pos.	-	-	Pos.	-	-	-
C-3	Pos.	-	-	Pos.	-	-	-
C-4	Pos.	-	-	Pos.	-	-	-
C-5	Pos.	-	-	-	-	Pos.	-
C-6	Pos.	-	-	-	-	-	-
C-7	-	-	-	Pos.	-	-	Pos.
C-8	Pos.	-	-	-	-	Pos.	-
C-8	Pos.	Pos.	-	Pos.	-	Pos.	-
C-9	Pos.	-	-	-	-	Pos.	-
C-10	Pos.	-	-	Pos.	-	Pos.	-
C-11	Pos.	-	-	Pos.	-	Pos.	-
C-12	Pos.	-	-	Pos.	-	-	-
% expr.	92,3	7,7 ^a	0	69,2	0	46,2 ^b	15,4

^a p < 0.09 using Fisher's Exact Test.

^b p = 0.01 using Fisher's Exact Test

*Primers designed for the common region of PDE4D identical for all isoforms

Table 9

Publically Available SNPS; SNP ID No. from NCBI Database

rs286155	rs40512	rs251726	rs2042315	rs1544791	rs1355099
rs286156	rs35386	rs1862589	rs918590	rs851284	rs1396473
rs2061250	rs35387	rs702556	rs918591	rs1396476	rs1369285
rs286150	rs27221	rs702554	rs918592	rs1508860	rs1435071
rs206789	rs27653	rs441391	rs1115372	rs1974850	rs1435070
rs1823062	rs26955	rs446883	rs1345782	rs2136203	rs1435083
rs1823063	rs26956	rs789615	rs1363862	rs2174994	rs991551
rs1445852	rs153031	rs401207	rs1423248	rs1508863	rs1154790
rs766119	rs185190	rs364917	rs1423246	rs1508859	rs1154789
rs956721	rs37762	rs404202	rs1862614	rs1508864	rs714291
rs248910	rs37761	rs440607	rs2194256	rs1396474	rs981760
rs248912	rs1423471	rs411255	rs889305	rs1543951	rs1369288
rs187481	rs27224	rs615429	rs2113071	rs2016324	rs977418
rs153152	rs1645013	rs789396	rs2113072	rs1995780	rs977417
rs27960	rs1423472	rs37684	rs966220	rs1508865	rs977416
rs27564	rs27220	rs1445893	rs966221	rs952110	rs1529843
rs27565	rs1423473	rs37685	rs719702	rs1533019	rs1529842
rs26948	rs149079	rs1086121	rs2113073	rs2117552	rs1435077
rs40131	rs149324	rs42222	rs2113074	rs1545069	rs1369287
rs26949	rs153067	rs37707	rs2113075	rs1545070	rs1017410
rs26950	rs40354	rs37708	rs1035512	rs973700	rs1017409
rs26954	rs26951	rs37709	rs1559277	rs1583434	rs1435076
rs26953	rs153029	rs789389	rs1981848	rs1347401	rs1435075
rs152324	rs27223	rs1423247	rs1544788	rs1949017	rs1435074
rs35385	rs27222	rs874768	rs1544790	rs723962	rs978455

rs1827340	rs159621	rs1504982	rs298084	rs298027	rs295972
rs1393083	rs159625	rs877745	rs298083	rs298028	rs295971
rs988364	rs1435072	rs877744	rs298073	rs298029	rs295970
rs1017408	rs173945	rs2164661	rs298072	rs298030	rs295969
rs2053155	rs256356	rs981230	rs298071	rs169868	rs295968
rs181923	rs185351	rs1437124	rs1421400	rs177077	rs295966
rs1546364	rs256355	rs746477	rs402874	rs298032	rs726652
rs173942	rs2067024	rs893191	rs434368	rs298033	rs295965
rs159616	rs256354	rs1992112	rs371011	rs298034	rs1307218
rs159620	rs173944	rs298102	rs298063	rs298035	rs1307217
rs1501641	rs256353	rs298101	rs298062	rs298042	rs893190
rs159619	rs986400	rs2164660	rs298061	rs298044	rs1111495
rs159614	rs1504981	rs298100	rs298060	rs298045	rs295961
rs159613	rs1120533	rs298098	rs298057	rs298046	rs295960
rs159612	rs256351	rs298096	rs298056	rs298048	rs295959
rs159611	rs190458	rs298095	rs1370230	rs298049	rs295958
rs194368	rs256352	rs298094	rs297975	rs298050	rs296410
rs661576	rs171745	rs298093	rs297974	rs298051	rs295957
rs299627	rs1157709	rs1362942	rs379578	rs298052	rs295956
rs159608	rs1910790	rs1362941	rs920190	rs298053	rs295955
rs159609	rs1910789	rs298091	rs1865962	rs190936	rs295954
rs159624	rs1504985	rs298090	rs298018	rs298017	rs295949
rs1159470	rs1008709	rs298089	rs298021	rs298016	rs295980
rs159622	rs1027747	rs298088	rs298022	rs298015	rs295979
rs256349	rs869685	rs298087	rs298023	rs298014	rs295978
rs256348	rs869686	rs1421401	rs298024	rs2053229	rs1154587
rs1501640	rs924880	rs298086	rs298025	rs295974	rs296406
rs600611	rs1504983	rs298085	rs298026	rs295973	rs296405

rs295948	rs294478	rs37575	rs1457111	rs171800	rs403695
rs295947	rs953302	rs37576	rs1824154	rs187716	rs403672
rs295946	rs294479	rs1876209	rs2112911	rs258110	rs372309
rs295945	rs697075	rs190486	rs1551564	rs258109	rs424839
rs295944	rs294481	rs447261	rs2034895	rs258108	rs370891
rs1395334	rs294482	rs1506558	rs2081092	rs258107	rs434183
rs295943	rs294483	rs1108916	rs2112910	rs665836	rs444552
rs1035321	rs702545	rs921942	rs918583	rs392901	rs433565
rs294494	rs294484	rs924998	rs1840838	rs383444	rs1445918
rs722923	rs294485	rs176705	rs1350298	rs662643	rs441817
rs294495	rs294486	rs1156029	rs1990985	rs670169	rs433161
rs294496	rs702544	rs1156028	rs1379297	rs525099	rs428059
rs294497	rs702543	rs931857	rs1817248	rs669240	rs434422
rs294498	rs159194	rs931856	rs244569	rs381755	rs427433
rs294499	rs40215	rs931855	rs244568	rs454702	rs391377
rs294500	rs291118	rs1506557	rs244567	rs443191	rs414746
rs294501	rs1506560	rs462930	rs244565	rs380118	rs187368
rs294503	rs37569	rs458953	rs185417	rs2168649	rs244593
rs295936	rs291119	rs174039	rs258128	rs371775	rs244592
rs1395336	rs37571	rs2174624	rs258127	rs378970	rs244591
rs1395337	rs1870077	rs2135480	rs258125	rs401013	rs244590
rs294492	rs159195	rs992726	rs1348710	rs427748	rs181736
rs159196	rs37572	rs294474	rs1348709	rs427740	rs193447
rs159197	rs37573	rs294475	rs1971061	rs378869	rs2028842
rs172362	rs167161	rs988827	rs1541673	rs1902609	rs2028841
rs37579	rs37574	rs988828	rs1541672	rs389324	rs1823068
rs721784	rs1506562	rs1350297	rs258112	rs387647	rs1823067
rs697076	rs291122	rs1457110	rs258111	rs377451	rs1823066

rs244588	rs35275	rs2014012	rs531105	rs27691	rs464311
rs168641	rs40125	rs37353	rs27184	rs35310	rs149108
rs2059175	rs35274	rs187645	rs1445951	rs26689	rs153980
rs2059174	rs244577	rs1809012	rs1947090	rs27187	rs153961
rs1118965	rs35267	rs187644	rs26708	rs1445948	rs1867725
rs154028	rs35266	rs153981	rs2112959	rs26687	rs153965
rs151802	rs39672	rs255652	rs1445953	rs166260	rs153966
rs244580	rs958851	rs255650	rs26709	rs149506	rs1988803
rs1457145	rs244576	rs255649	rs26710	rs27722	rs467300
rs244579	rs244575	rs2194210	rs28055	rs26695	rs1664886
rs255812	rs244573	rs255648	rs26711	rs27773	rs1867724
rs154029	rs35258	rs255647	rs27723	rs1471429	rs1445947
rs185333	rs35259	rs154221	rs27185	rs1471430	rs42470
rs35289	rs40121	rs256752	rs27695	rs26705	rs1423308
rs35288	rs35261	rs256120	rs1445954	rs28054	rs27174
rs35287	rs35264	rs255635	rs27549	rs26703	rs168834
rs35286	rs40122	rs185325	rs455969	rs27898	rs27727
rs35285	rs35265	rs26686	rs26712	rs722010	rs27172
rs35284	rs35255	rs1031197	rs1867711	rs27957	rs676449
rs35283	rs721826	rs1031198	rs1867712	rs26702	rs27186
rs35282	rs244570	rs27183	rs26713	rs27548	rs2112957
rs35281	rs27171	rs28044	rs26714	rs26701	rs1023814
rs35280	rs1824159	rs27182	rs27547	rs27188	rs27175
rs35279	rs27170	rs545611	rs26715	rs27189	rs1445950
rs35278	rs27169	rs649476	rs27949	rs149084	rs2021384
rs40126	rs27168	rs1664896	rs26700	rs153968	rs736736
rs35277	rs2013979	rs149106	rs1306348	rs464787	rs745813
rs35276	rs889231	rs1374028	rs35309	rs153978	rs889229

rs1077978	rs1353749	rs2055295
rs2081106	rs1391651	rs1391648
rs1559252	rs1391650	rs2055298
rs2054443	rs1391649	rs1472456
rs922437	rs1391652	rs1553114
rs922436	rs950446	rs1542842
rs922435	rs950447	rs1498611
rs922434	rs1498599	rs1532520
rs716908	rs1498601	
rs1971940	rs1498609	
rs1559251	rs1498608	
rs1345791	rs1553113	
rs1345792	rs1353748	
rs1345793	rs1498606	
rs1105577	rs1353747	
rs1960	rs1006431	
rs1824788	rs1948651	
rs1862563	rs1498605	
rs1551939	rs1498604	
rs1038080	rs1498603	
rs997421	rs1995166	
rs1014317	rs1498602	
rs2059191	rs1077183	
rs1551938	rs1078368	
rs1186170	rs1874857	
rs986067	rs1874858	
rs954740	rs1909294	
rs1363882	rs1546221	

Table 10
New SNP's identified by deCODE

Position in patent	Variation	AA Change	Exon		
				1268007	A/G
732790	G/T			1268187	C/T
735966	C/A			1268553	A/G
736226	A/G			1272669	G/A
736516	C/T			1272910	A/G
850001	G/A			1273023	G/A
852776	A/C			1273220	A/G
853079	G/T			1273240	A/G
853575	C/A			1273543	C/T
856468	A/G			1288439	G/A
860845	A/G			1289730	T/A
870924	A/G			1290176	G/A
1027267	T/C			1293745	T/C
1027643	T/G			1344605	A/G
1027757	T/C			1344864	G/A
1028146	T/A			1345135	C/G
1037657	A/C			1345286	A/G
1044016	G/A			1346112	C/T
1044045	C/T			1352976	A/T
1254737	T/C			1354291	T/C
1254849	T/C			1354377	C/T
1255763	G/T			1354554	C/A
1257206	A/G			1354675	T/C
1258161	T/C			1355114	T/C

1355693	A/G	1575634	A/T		
1357081	A/G	1580088	G/A		
1362985	T/G	1581078	G/A		
1363021	C/T	1582418	T/A		
1363827	C/T	1584580	A/C		
1363911	G/A	1585955	G/T		
1364061	C/T	1590608	T/C		
1364066	T/A	1590672	A/G		
1367904	A/G	1590673	G/T		
1368193	T/C	1590837	G/A		
1368217	G/C	1590936	C/A		
1373349	C/T	1591011	G/A		
1373384	A/G	1591047	C/T		
1373415	T/C	1591306	C/A	Pro->Thr	D1
1373979	T/G	1591583	T/C		
1376149	G/A	1594788	C/A		
1384931	A/C	1594994	G/A		
1385093	A/T	1601831	C/T		
1385107	G/A	1636902	T/C		
1385445	T/C	1638550	A/C	Lys->Thr	exon 4
1391418	G/C	1640663	T/C		
1409210	C/A	1641954	C/T		
1414804	C/T	1641960	C/T		
1428284	T/C	1653881	G/A		
1431800	A/T	1655748	G/A		
1449904	A/T				
1574301	C/G				
1574615	C/T				

While this invention has been particularly shown and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule comprising a phosphodiesterase 4D gene, or a fragment or variant thereof.
- 5 2. The isolated nucleic acid molecule of Claim 1, wherein the phosphodiesterase 4D gene has the nucleotide sequence of SEQ ID NO:1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof.
3. A nucleic acid encoding a polypeptide having an amino acid sequence selected
10 from the group consisting of SEQ ID NOs: 2-10, 12 or 14.
4. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof, and the complement thereof.
- 15 5. An isolated nucleic acid molecule which hybridizes under high stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof, and the complement thereof.

6. An isolated nucleic acid molecule which hybridizes under high stringency conditions to a nucleotide sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NOs: 2-10, 12 or 14.
7. A method for assaying the presence of a first nucleic acid molecule in a sample, comprising contacting said sample with a second nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof, and the complement thereof, under high stringency conditions.
8. A vector comprising an isolated nucleic acid molecule selected from the group consisting of: SEQ ID NO: 1, the complement of SEQ ID NO: 1 SEQ ID NOs: 2-10, 12 or 14, operatively linked to a regulatory sequence; wherein the nucleic acid molecule may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof.
9. A recombinant host cell comprising the vector of Claim 8.
10. A method for producing a polypeptide encoded by an isolated nucleic acid molecule, comprising culturing the recombinant host cell of Claim 9 under conditions suitable for expression of said nucleic acid molecule.
11. An isolated polypeptide encoded by a phosphodiesterase 4D gene, or a fragment or variant of said polypeptide.
12. The isolated polypeptide of Claim 11, wherein the phosphodiesterase 4D gene has the sequence of SEQ ID NO: 1 which may optionally comprise at least one

polymorphism as shown in Table 9, 10 or combination thereof, or the complement thereof.

13. The isolated polypeptide of Claim 11, wherein the polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-10, 12 or 14.
14. An isolated polypeptide comprising an amino acid sequence which is greater than about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-10, 12 or 14.
15. A fusion protein comprising an isolated polypeptide of Claim 11.
16. An antibody, or an antigen-binding fragment thereof, which selectively binds to a polypeptide of Claim 11.
17. An antibody, or an antigen-binding fragment thereof, which selectively binds to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-10, 12 or 14, or to a fragment or variant of said amino acid sequence.
18. A method for assaying the presence of a polypeptide encoded by an isolated nucleic acid molecule according to Claim 1 in a sample, comprising contacting said sample with an antibody which specifically binds to the encoded polypeptide.
19. A method of diagnosing a susceptibility to stroke in an individual, comprising detecting a polymorphism in phosphodiesterase 4D gene, wherein the presence of the polymorphism in the gene is indicative of a susceptibility to stroke.

20. A method of diagnosing a susceptibility to stroke, comprising detecting an alteration in the expression or composition of a polypeptide encoded by phosphodiesterase 4D gene in a test sample, in comparison with the expression or composition of a polypeptide encoded by phosphodiesterase 4D gene in a control sample, wherein the presence of an alteration in expression or composition of the polypeptide in the test sample is indicative of a susceptibility to stroke.
21. The method of Claim 20, wherein the alteration in the expression or composition of a polypeptide encoded by phosphodiesterase 4D gene comprises expression of a splicing variant polypeptide in a test sample that differs from a splicing variant polypeptide expressed in a control sample.
22. A method of identifying an agent which alters activity of a polypeptide of Claim 11, comprising:
- a) contacting the polypeptide or a derivative or fragment thereof, with an agent to be tested;
 - b) assessing the level of activity of the polypeptide or derivative or fragment thereof; and
 - c) comparing the level of activity with a level of activity of the polypeptide or active derivative or fragment thereof in the absence of the agent,
- wherein if the level of activity of the polypeptide or derivative or fragment thereof in the presence of the agent differs, by an amount that is statistically significant, from the level in the absence of the agent, then the agent is an agent that alters activity of the polypeptide.

23. An agent which alters activity of a polypeptide encoded by phosphodiesterase 4D gene, identifiable according to the method of Claim 22.
24. An agent which alters activity of a polypeptide encoded by phosphodiesterase 4D gene, wherein the agent is selected from the group consisting of: a
5 phosphodiesterase 4D gene receptor; a phosphodiesterase 4D gene binding agent; a peptidomimetic; a fusion protein; a prodrug; an antibody; and a ribozyme.
25. A method of altering activity of a polypeptide encoded by phosphodiesterase 4D gene, comprising contacting the polypeptide with an agent of Claim 24.
- 10 26. A method of identifying an agent which alters interaction of the polypeptide of Claim 11 with a phosphodiesterase 4D gene binding agent, comprising:
- a) contacting the polypeptide or a derivative or fragment thereof, the binding agent and with an agent to be tested;
 - b) assessing the interaction of the polypeptide or derivative or
15 fragment thereof with the binding agent; and
 - c) comparing the level of interaction with a level of interaction of the polypeptide or derivative or fragment thereof with the binding agent in the absence of the agent,
- 20 wherein if the level of interaction of the polypeptide or derivative or fragment thereof in the presence of the agent differs, by an amount that is statistically significant, from the level of interaction in the absence of the agent, then the agent is an agent that alters interaction of the polypeptide with the binding agent.
27. An agent which alters interaction of a phosphodiesterase 4D gene polypeptide
25 with a phosphodiesterase 4D gene binding agent, identifiable according to the method of Claim 26.

28. An agent which alters interaction of a phosphodiesterase 4D gene polypeptide with a first phosphodiesterase 4D gene binding agent, selected from the group consisting of: a phosphodiesterase 4D gene receptor; a second phosphodiesterase 4D gene binding agent; a peptidomimetic; a fusion protein; a
5 prodrug; an antibody; and a ribozyme.
29. A method of altering interaction of a phosphodiesterase 4D gene polypeptide with a phosphodiesterase 4D gene binding agent, comprising contacting the phosphodiesterase 4D gene polypeptide and/or the phosphodiesterase 4D gene binding agent with an agent of Claim 28.
- 10 30. A method of identifying an agent which alters expression of phosphodiesterase 4D gene, comprising the steps of:
- a) contacting a solution containing a nucleic acid of Claim 1 or a derivative or fragment thereof with an agent to be tested;
 - 15 b) assessing the level of expression of the nucleic acid, derivative or fragment; and
 - c) comparing the level of expression with a level of expression of the nucleic acid, derivative or fragment in the absence of the agent,
- 20 wherein if the level of expression of the nucleotide, derivative or fragment in the presence of the agent differs, by an amount that is statistically significant, from the expression in the absence of the agent, then the agent is an agent that alters expression of phosphodiesterase 4D gene.
31. An agent which alters expression of phosphodiesterase 4D gene, identifiable according to the method of Claim 30.
- 25 32. A method of identifying an agent which alters expression of phosphodiesterase 4D gene, comprising the steps of:

- 5 a) contacting a solution containing a nucleic acid comprising
 the promoter region of phosphodiesterase 4D gene operably
 linked to a reporter gene, with an agent to be tested;
 b) assessing the level of expression of the reporter gene; and
 c) comparing the level of expression with a level of expression of the
 reporter gene in the absence of the agent,

 wherein if the level of expression of the reporter gene in the presence of the
 agent differs, by an amount that is statistically significant, from the level of
 expression in the absence of the agent, then the agent is an agent that alters
10 expression of phosphodiesterase 4D gene.

33. An agent which alters expression of phosphodiesterase 4D gene, identifiable
 according to the method of Claim 32.

34. A method of identifying an agent which alters expression of phosphodiesterase
 4D gene, comprising the steps of:

- 15 a) contacting a solution containing a nucleic acid of Claim 1
 or a derivative or fragment thereof with an agent to be
 tested;
 b) assessing expression of the nucleic acid, derivative or fragment;
 and
20 c) comparing expression with expression of the nucleic acid,
 derivative or fragment in the absence of the agent,

 wherein if expression of the nucleotide, derivative or fragment in the presence
 of the agent differs, by an amount that is statistically significant, from the
 expression in the absence of the agent, then the agent is an agent that alters
25 expression of phosphodiesterase 4D gene.

35. The method of Claim 34, wherein the expression of the nucleotide, derivative or
 fragment in the presence of the agent comprises expression of one or more

splicing variant(s) that differ in kind or in quantity from the expression of one or more splicing variant(s) the absence of the agent.

36. An agent which alters expression of phosphodiesterase 4D gene, identifiable according to the method of Claim 34.
- 5 37. An agent which alters expression of phosphodiesterase 4D gene, selected from the group consisting of: antisense nucleic acid to phosphodiesterase 4D gene; a phosphodiesterase 4D gene polypeptide; a phosphodiesterase 4D gene receptor; a phosphodiesterase 4D gene binding agent; a peptidomimetic; a fusion protein; a prodrug thereof; an antibody; and a ribozyme.
- 10 38. A method of altering expression of phosphodiesterase 4D gene, comprising contacting a cell containing phosphodiesterase 4D gene with an agent of Claim 37.
- 15 39. A method of identifying a polypeptide which interacts with a phosphodiesterase 4D gene polypeptide, comprising employing a two yeast hybrid system using a first vector which comprises a nucleic acid encoding a DNA binding domain and a phosphodiesterase 4D gene polypeptide, splicing variant, or fragment or derivative thereof, and a second vector which comprises a nucleic acid encoding a transcription activation domain and a nucleic acid encoding a test polypeptide, wherein if transcriptional activation occurs in the two yeast hybrid system, the
- 20 test polypeptide is a polypeptide which interacts with a phosphodiesterase 4D polypeptide.
40. A phosphodiesterase 4D gene therapeutic agent selected from the group consisting of: a phosphodiesterase 4D gene or fragment or derivative thereof; a polypeptide encoded by phosphodiesterase 4D gene; a phosphodiesterase 4D
- 25 gene receptor; a phosphodiesterase 4D gene binding agent; a peptidomimetic; a

fusion protein; a prodrug; an antibody; an agent that alters phosphodiesterase 4D gene expression; an agent that alters activity of a polypeptide encoded by phosphodiesterase 4D gene; an agent that alters posttranscriptional processing of a polypeptide encoded by phosphodiesterase 4D gene; an agent that alters
5 interaction of a phosphodiesterase 4D gene with a phosphodiesterase 4D gene binding agent; an agent that alters transcription of splicing variants encoded by phosphodiesterase 4D gene; and a ribozyme.

41. A pharmaceutical composition comprising a phosphodiesterase 4D gene therapeutic agent of Claim 40.
- 10 42. The pharmaceutical composition of Claim 41, wherein the phosphodiesterase 4D gene therapeutic agent is an isolated nucleic acid molecule comprising a phosphodiesterase 4D gene or fragment or derivative thereof.
43. The pharmaceutical composition of Claim 41, wherein the phosphodiesterase 4D gene therapeutic agent is a polypeptide encoded by the phosphodiesterase
15 4D gene.
44. A method of treating stroke in an individual, comprising administering a phosphodiesterase 4D gene therapeutic agent to the individual, in a therapeutically effective amount.
45. The method of Claim 44, wherein the phosphodiesterase 4D gene therapeutic
20 agent is a phosphodiesterase 4D gene agonist.
46. The method of Claim 45 wherein the phosphodiesterase 4D gene therapeutic agent is a phosphodiesterase 4D gene antagonist.

47. A transgenic animal comprising a nucleic acid selected from the group consisting of: an exogenous phosphodiesterase 4D gene and a nucleic acid encoding a phosphodiesterase 4D gene polypeptide.
48. A method for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid, comprising:
- 5 a) contacting said sample with a nucleic acid comprising a contiguous nucleotide sequence which is at least partially complementary to a part of the sequence of said phosphodiesterase 4D gene nucleic acid under conditions appropriate for hybridization, and
- 10 b) assessing whether hybridization has occurred between a phosphodiesterase 4D gene nucleic acid and said nucleic acid comprising a contiguous nucleotide sequence which is at least partially complementary to a part of the sequence of said phosphodiesterase 4D gene nucleic acid.
- 15 49. The method of Claim 48, wherein said nucleic acid comprising a contiguous nucleotide sequence is completely complementary to a part of the sequence of said phosphodiesterase 4D gene nucleic acid.
50. The method of Claim 48, comprising amplification of at least part of said phosphodiesterase 4D gene nucleic acid.
- 20 51. The method of Claim 48, wherein said contiguous nucleotide sequence is 100 or fewer nucleotides in length and is either: a) at least 80% identical to a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; b) at least 80% identical to the complement of a contiguous sequence of
- 25 nucleotides in SEQ ID NO: 1 which may optionally comprise at least one

polymorphism as shown in Table 9, 10 or combination thereof; or c) capable of selectively hybridizing to said phosphodiesterase 4D gene nucleic acid.

52. A reagent for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid, said reagent comprising a nucleic acid comprising a
5 contiguous nucleotide sequence which is at least partially complementary to a part of the nucleotide sequence of said phosphodiesterase 4D gene nucleic acid.
53. The reagent of Claim 52, wherein the nucleic acid comprises a contiguous nucleotide sequence which is completely complementary to a part of the nucleotide sequence of said phosphodiesterase 4D gene nucleic acid.
- 10 54. A reagent kit for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid, comprising in separate containers:
- a) one or more labeled nucleic acids comprising a contiguous
nucleotide sequence which is at least partially complementary to a
part of the nucleotide sequence of said phosphodiesterase 4D gene
15 nucleic acid, and
- b) reagents for detection of said label.
55. The reagent kit of Claim 54, wherein the labeled nucleic acid comprises a contiguous nucleotide sequences which is completely complementary to a part of the nucleotide sequence of said phosphodiesterase 4D gene nucleic acid.
- 20 56. A reagent kit for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid, comprising one or more nucleic acids comprising a contiguous nucleotide sequence which is at least partially complementary to a part of the nucleotide sequence of said phosphodiesterase 4D gene nucleic acid, and which is capable of acting as a primer for said phosphodiesterase 4D gene
25 nucleic acid when maintained under conditions for primer extension.

57. The use of a nucleic acid which is 100 or fewer nucleotides in length and which is either: a) at least 80% identical to a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; b) at least 80% identical to the complement of a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; or c) capable of selectively hybridizing to said phosphodiesterase 4D gene nucleic acid, for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid.
58. The use of a nucleic acid which is 100 or fewer nucleotides in length and which is either: a) at least 80% identical to a contiguous sequence of nucleotides in SEQ ID NO: 1; b) at least 80% identical to the complement of a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; or c) capable of selectively hybridizing to said phosphodiesterase 4D gene nucleic acid, for assaying a sample for the presence of a phosphodiesterase 4D gene nucleic acid that has at least one nucleotide difference from SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof.
59. The use of a nucleic acid which is 100 or fewer nucleotides in length and which is either: a) at least 80% identical to a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; b) at least 80% identical to the complement of a contiguous sequence of nucleotides in SEQ ID NO: 1 which may optionally comprise at least one polymorphism as shown in Table 9, 10 or combination thereof; or c) capable of selectively hybridizing to said phosphodiesterase 4D gene nucleic acid, for diagnosing a susceptibility to stroke.

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Genetic map

Combined map - cM

Physical map - Mb

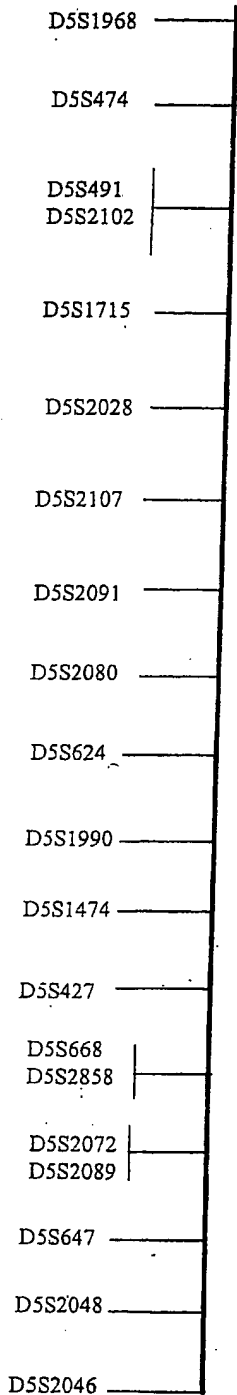


Fig. 2A

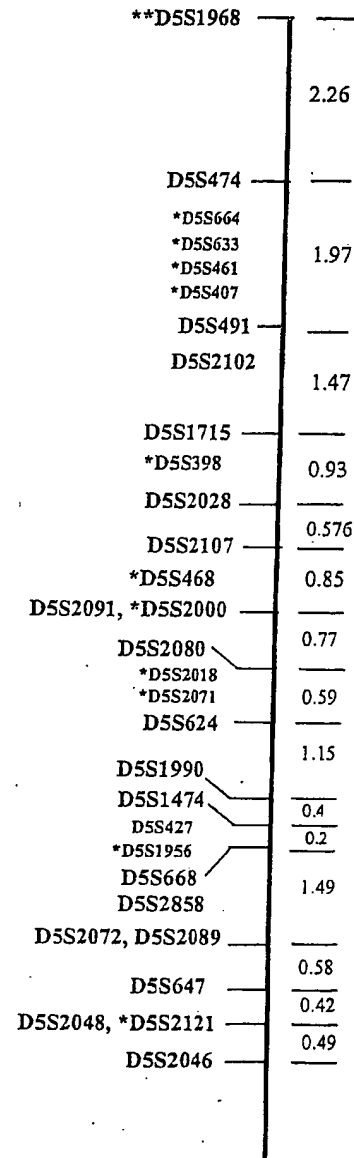


Fig. 2B

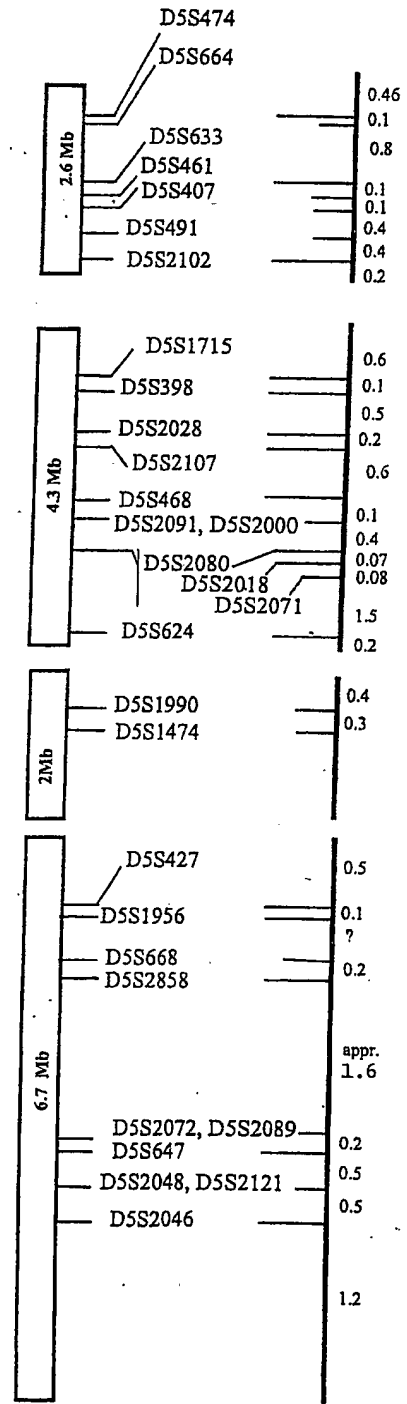


Fig. 2C

*Markers only assigned in physical map

**Marker in blue - only assigned in genetic map

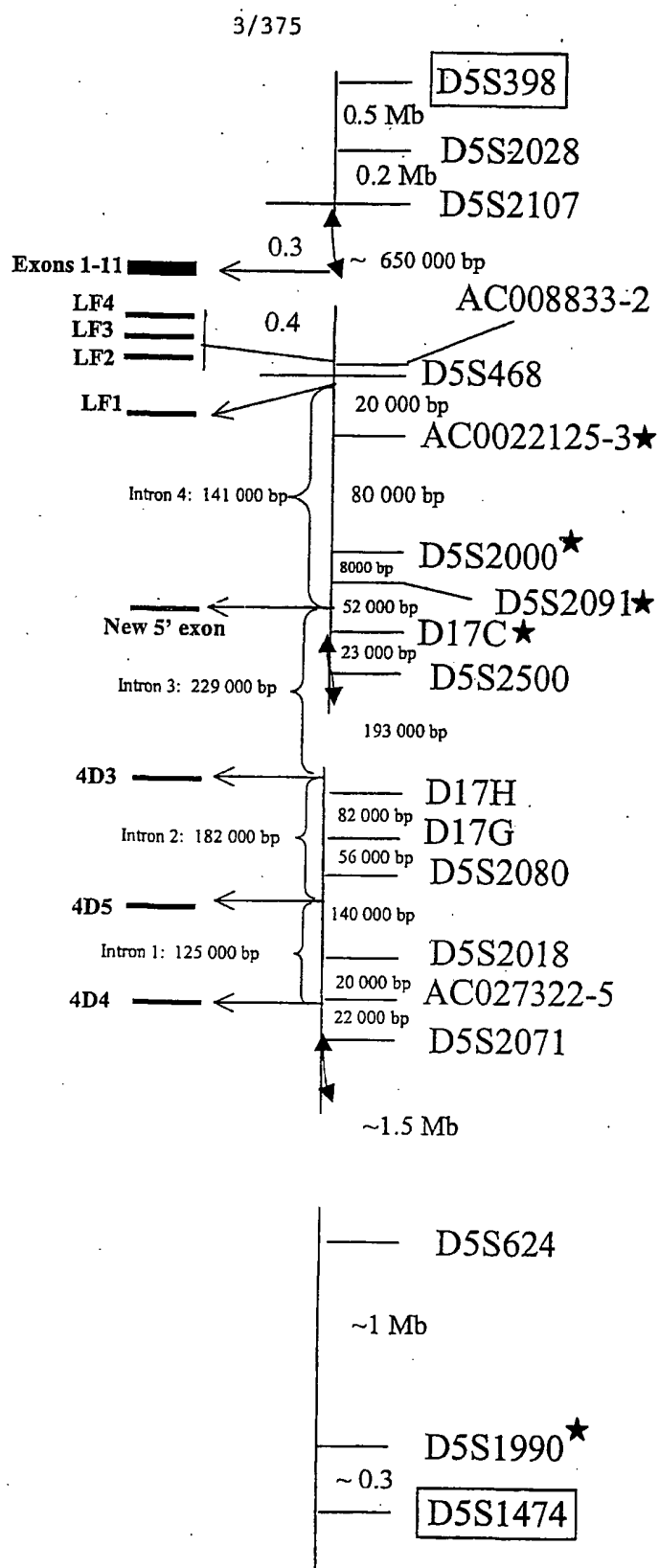


Fig. 3

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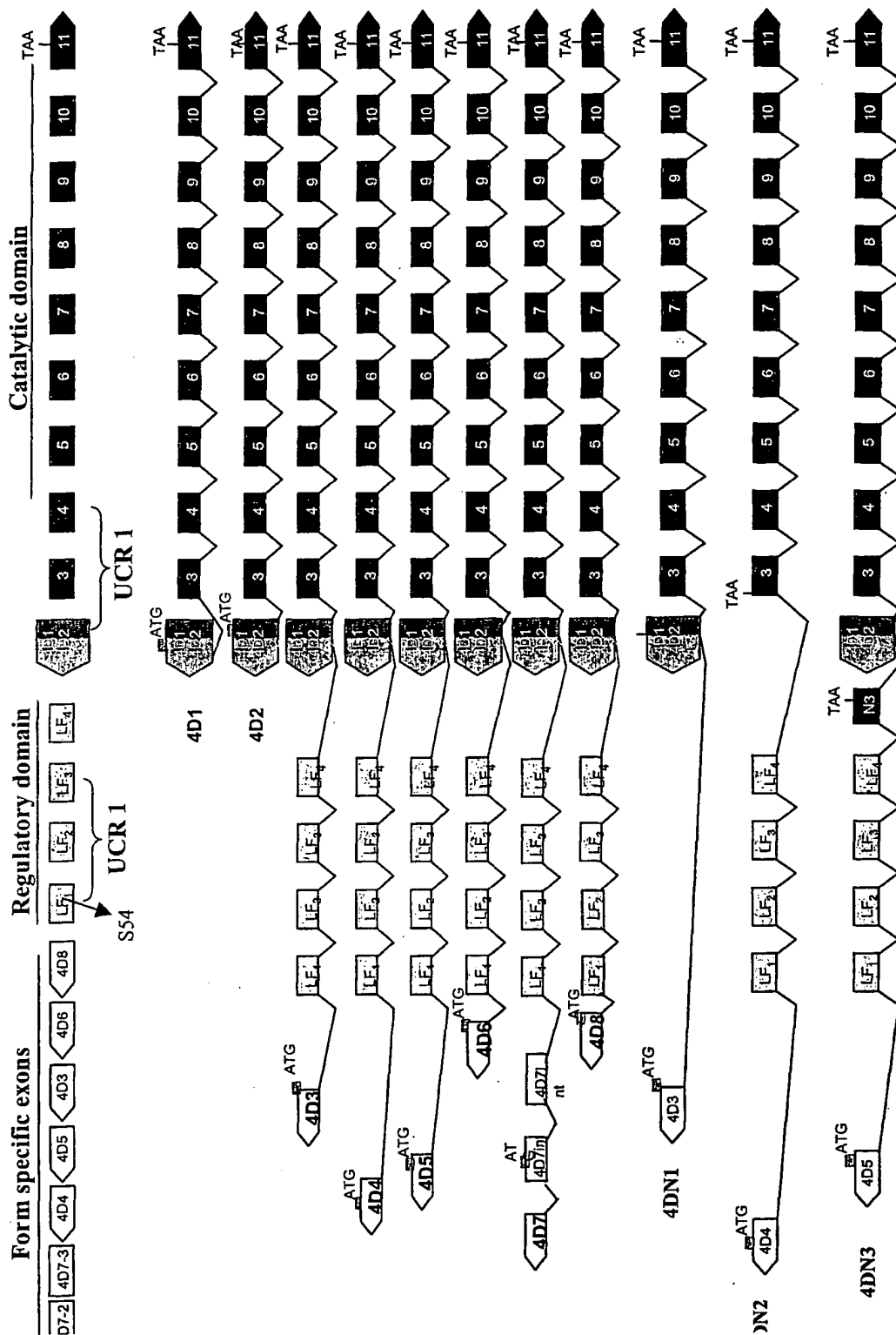
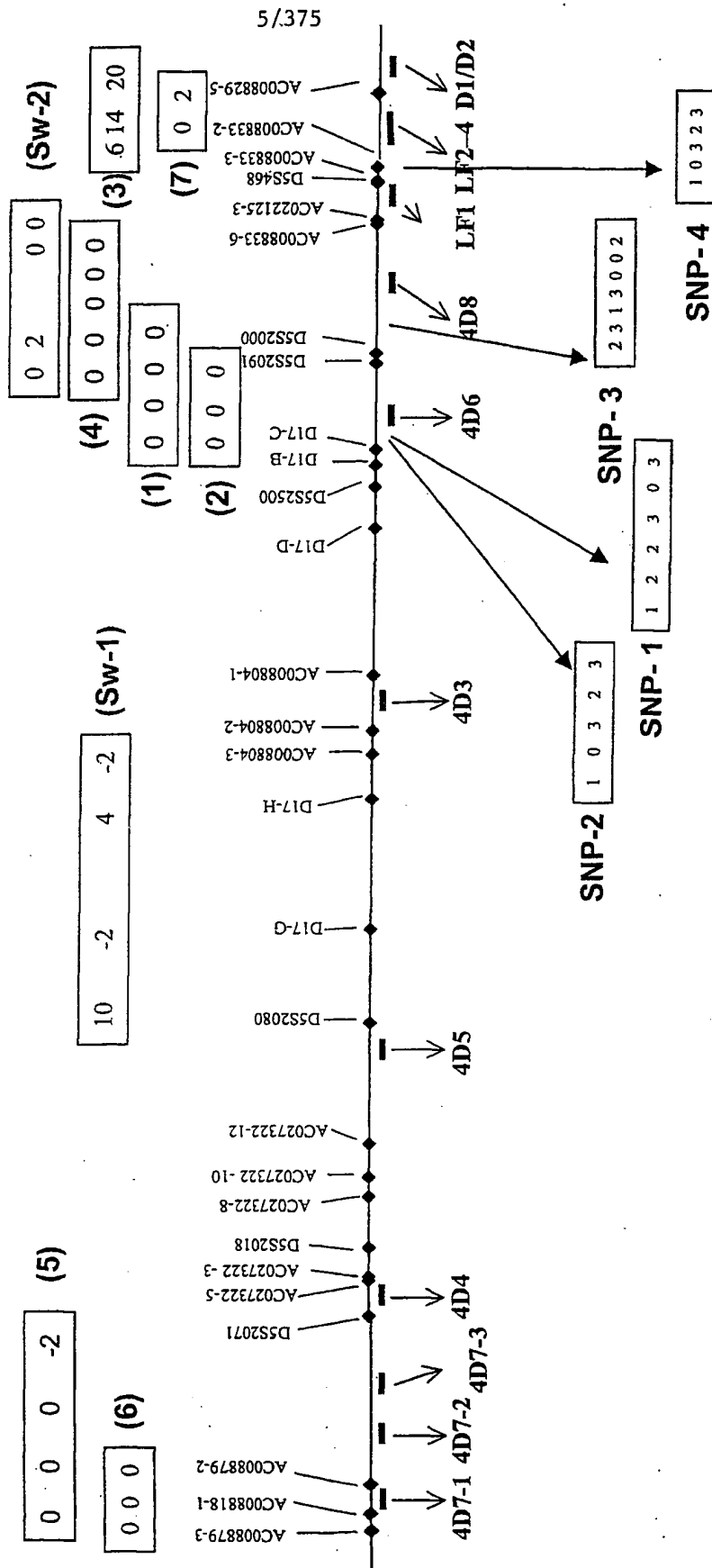


Fig. 4

Position of selected haplotypes within gene

microsatellite haplotypes



SNP haplotypes

Fig. 5

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>Contig_2 (1,1691140)

CATTTTTTGAAGAACATCTGAAAGACAAAATGGGGAATGGCGAGTCTGTCTAATAAACCATTTTTGAGAAAACCTGGATA
TTCATATAAAGAAAGTATAAAGAGGACTGTTATGTTGCACAATACACAAAAATCAACTCAAAATAAATTAATGACCTAAA
CTTAATATCAGAAATGATGAACTTTTAAAAGAAAACATAGGGTGAATGTATGTTTAGGGAACTCAAATTTATGTTCA
CAGGTTGCAAAAAGAAAAATATGAGAAAAACATAGAGGAAAATGATTCTGCCAATAAAGTGAGTTGGAAATAATTTTTTC
TGTTTTTCACAAAATCATTGCTAACAAAAGCAAAAACAAGTGTGGGACTATAGAAAACCTGATGAGCTTCTGCATAGGAA
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GGGCAAGAACCTGAATAGATGTTTTCTGAATAGAAGACATGAATTTGACTACCAGGTAAAAGAAAAGTTCTCAACATA
CCTAATCATCAAGAAAATGTACATTAAAACCTCACTGAGATATCTTCTCCACTCTAATTGGAATTAATGTTACAAAAAG
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GAATTTAAGTCAGTATACACACTATGAAAAATAGTTGGAGTTTGCTCAAAAAATAAAATACAACCTATCATTGCTGTAG
TAATCCCACCACTGAATATACATTTTAAGAAAATGAAATCAGTATATTGAAGAGATACGTGAAATCCTACATTTCTTGA
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TGATGTGTAGTCTGAATCCTGGCTAAGTATAAACCTTTTATTTTTTATACCTGTTCTTAGTGAAAATGAACTGTGACT
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AGATTTTTTATGTGAACTACTTGTGAGAGATCTTAACAATTTGTAGTTAGAGAAAGCACTATTATATCATTTGGAAATG
CAAGAAACAAGTTACCTTTGGGGCAACAGAGGCCCTTGTCAATTTCTCAAAAGAAGGAAGCATCAGCATTGTTGATGATG
ATGTTGAGATTGTAGAAATGATGAAGGTGAAAAGTTATTCTAGCTTATGTTTAGCAAAATGAAATGAACCCAAATAAT
AAAACAGTTACAACATGTAATCTCTTTGGGAGAAAAAAGATAGAATGCTAATGTCTTTCAGAACTTCTTTAAACCA
GAACCTTAAAAAAGAGAAGCTTTTAAAAAATCATAATAGTTTATGATCTTGAAGGGTTTAAAGTATTTGATGAAGA
TGTCTTTTGAATTTATTTGTAGGTCTTCTTGTGTATTTAAAAGCTAAGTTATCTTGAATCATTTTTTTTCTATACCTTT
GTCAGTAACCTCTTAGTGATGAAATAAAAAAGATTAGGTAATCATCCAGCAATGGGGAAGAAAGTTAAGGAACAAAGAGC
TCAGATTAACTAGTTTTTGAATCTAAGCATTCTGTCATGAATTTGAATCATGGAAAACAAAATGTAGCACTCCAACA
TTTGATGCAAACTAAAAGTGGAACTGCTTTGATATTTGAATGAATTGAAAATAAATAACATCCTTGGAACTGTAT
GTAAAGAAGGACTTCACAAGTATTATAGATACCCCAACCTCAGCCCTTTCCCATGTATCTCTTTGATCACAATCCCTA
CCTCATAGATCACCCATGTGCTGAACACTTTCAGTTCTGTATCTTTCATTCTAGATCTCCTGAACCTCAAGATCAGAATAT
CTTTCTGACTTCTGACTGTGTATTCTGATGTTATACAAGAACCTCAGCTCAAACCTCAGTATTCCTTAAACCATTGTT
TTTGAACCTTTATGTTGGATGTGAAATCTGTATTGTAGAATAACATTAAGAAAGAAAGAAATAGTATGCAAAATATCAG
AGTGCATTGTATGTAGCAAGAGTAGGTATTTTCGTAAACTTTTTGTTTTAATTAAACACATATATATTATTATGTCAG
TGTAATATGATTTCTTAATTTTGATTAAGTGTAGTGAGGATGTGGATAAATTGGAACCTCTGTACATTACTGGTGGGA
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GAAATTTCACTTTTAGATGTACACCCAAAGAAATGAGAACATATGTTTACACAGCAACTTGTACACAATGTTTCATAG
CAGCAATTACTCAGAAGAGCCAAAAGGTGGAACAACTGAAATGTCCATCAAGTGATGAAGCAGTAAAATGTAGTATATC
CGTACAATGAAATATTCAGCCATAAAAAGGAATGCAATGTTGTTGCATGCTACAACAACCTGGATGAATCTTGGAAACA
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GGGGTTGTGGGGAGGAGAGAATGGGAAGGTGACAAATGTTCTGGATTAGATAATAGGGATGGGTATAACTTAGTGACT
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AAATTTGACTTTAGGAGTTAAAAAGAATATAGTATCTCAATGAAAATTTGCTGGATAGGATTAGGGGTAGATTAGAC
ACTCCAGAAGTTAAAGATCAGTGAGCTTGAATACACACAATGAGAGCTAGTCTAAACAAGACAGAGAGAAAAAGAA
CAAAACAACCTCCCAACAACAAAAAAGCAAAACCAAAATACAGCCTCAATGACCTATGGGAAATATTTAGC
AGTCTAATATACATGTAATTGGAATCCCTGAAGGAGGAGGGGGTAGAATGTATCTTTTTTTGTCCCCTATGACTGCTG
TTAAGATTTTATTATTGATTTTTTAGGAATTGCATTATATCTGGTGTGGTTGTTTAAACAGAGGTATAGCTTATCAACC

Fig. 6.1

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AATGGTGGAGCTAAATAGAACTTGAAGTACTTATGGATGCACAGAATCTAAGATGGCCCCCAATTTTCCTGCTAC
CTTGTACCCCTTGAGTATATGTGGGACCTGTTACTTGTCTTAACCAATAAAATCTCACACCAGTTAGAATGGTGATTAT
TAAAAAGTCAGGAAACAACGGATGCTGGAGAGGATGTGGAAAAATAGGAACGCTTTTACACTGTTGGTGGAAGTGTA
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CAGGGAATACTATGCAGCTATAAAAAATGACGAGTTCATGTCTTTGACGGGACATGGATGAAGCTGGAAACCATCATT
CTCAGCAAATATCACAAGAACAGAAAACCAAACACCACATCTTCTCACTCATAAGTGGCAGTTGAACAATGAGAACAC
ATGGACACAAGGCAGGGAACATCACACACCGGGGCTGTGTGGGGGGTGGGGGAGGGGATAGCATTAGGAGA
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TACCTGTGTTACAAACCTGCACGTTCTGTACATGTACCCAGAACTTAAAGTATAATAATAATAAATAACATGTATGT
CAAGGTGACATGTAATTAAGCAAAGCTCAGTAAATTTAAATGATTGAAATTTGTAAGTTTACTAAGTTTCTGACCGCATGT
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GAAAGTCTTTTCAAGAAATAGAAGATGAGGGAATATTTCTGAACCATTTTATGAGGCCAGTATTGACATGGGTAATAA
AACCAACAAATACATTACACAAAAAATTTGTAGCACATGATATCCCTGATAAAACCAATGCAAAAATACATTAAATTT
GCAAAATGAATGCAGCAGTAGATAAAAAGGACAATAATACATCATGGCCAAGTAGGGTTTATCCAGCAAGGTAAGACT
GGTTTAAACATCTAAATCAATCAGTATAATTCATCATATCGATAGGATGAAGGAAAAAACTCATGTGACCATCTCAAC
GATTGCAGAAATGTATGTGACAATATTCACACCCATTAATGATAAAAAATGTTAAATACATTACAATAAGAAGAAAT
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CCTGTTTTTAAAGACTTACTATAAAATCTTACTTTTCAAGGTGTGGTATTGGTATCTTACTGTAAAGTCTTCTGTAAAGTA
TATTGATATTTAGTGTGGTGTGGCATAAGGATAGATATTTAGGCCTATGGAATAGAATAGAGGGTCCAATAGTAGATT
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TCCCCCTACCCCGACCCCAACAGTCCCGGTGTGTGATGTTCCCTTCTGTGTCAATGTGTTCTCATTGTTCAATT
CCCACCTATGAGTGGCAACATGTGGTGTGTTTTGTTTTGTCCTTGAGATAGTTTGTGAGAATGATGGTTTTCCAGTTTC
ATCCATGTCCCTACAAAGCACATGAACCTATTATTTTTCATGGCTGCATAGTATTCCTGGTGTATAGTGCCACATTTT
CTTAATCCAGTCTATCACTGATGGACATTTGGGTTGGTTCCAAGTCTTTGCTATTGTGAATAGTGCCCTCAATAAACATA

Fig. 6.2

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CGTGTGCATGTGTCTTTATAGCAGCATGATTTATAATCCTTTGTGTATATACCCAGTAATGGGATGGCTGGGTCAAATG
GTATTTCTAGTTCTAGATCCTTGAGGAATCGCCACACTGTCTTCCACAATGGTTGAACCAAGTTTACAGTCCCACCAACA
GTGTAAAAGCATTTCCTATTTCTCCACATCCTCTCCAGCACCTGTTGTTTCTGACTTTTAAATGATCGCCATTCTAACT
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TCCCATCCTGTAGGTTGCTTGTTCACCTCTGATGGTAGTTTCTTTTGTCTGTGCAGAAGCTCTTTAGTTTAAATAGATCCT
GTTTGTCAATTTTGGCTTTTGTTCCTTGTCTTTTGGTGTTTTAGACATGAAGTCCTTGCCCATGCCATGTCTCTGAATG
GTATTGCCTAGGTTTTCTCTAGGGTTTTATGGTTTTAGGTCTAGCATTTAAGTCTTTAATCCATCTTGAATTAATTT
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TAGGGAATCATTTCCCCATTTCTTGTTTTTGTGAGGTTTGTCAAAGATCAGGTAGTTGTAGATATGTGGCATTATTTCT
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CCTCGATGGTCTTTACAATTTGGCATGATTTTGCAGTGGCTGGTACCCTATGTTCTTTCCATGTTTAGTGCTTCTTCTC
AGGAGCTCTTTTAGGGCAGGCTGGTGGTGACAAATCTCTCAGCATTGCTTGTCTGTAAAGGATTTTATTTCTCCTT
CACTTATGAAGCTTAGTTTGGCTGGATATGAAATCTGGGTTGAAATCTTTTCTTCCAGGAATGTTGAATATTGGTCC
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TAGTTTGTATCATCTGAAGCCTTCTGCTCTCAACTCGTCAAAGTCACTTCCATCCAGCTTTGTTCTGTTGCTGCTGAGG
AGCTGCGTTCTTTGGAGGAGGAGAGGTGCTCTGATTTTTTAGAGTTTCCAGTTTTTCTGCTCTGTTTTCCCCCATCTT
TGTGGTTTTATTACCTTTGGTCTTTGATGATGGTGACGTACAGATGGGGTTTTGGTGTGGATGTCCTTTCTGTTTTGT

Fig. 6.3

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AGTTTTCTTCTAACAGTTAGGACCCTCAGCTGCAGTCTGTTGGTGTGTTGCTGGAGGTCCACTCCAGACCCTGTTTGC
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GTTTTTCTCAGAGGAGTACCCGGCCATGTGAGGTGTCACTCAGCCCCCTACTGCGGGGTGCTCCAGTTAAGCTACTC
GGGAGTCAGGGACCCACTTGAGGAGGCAGTCTGTCCATTCTCAGATCTCAAGCTGCATGCTGGGAGAACCCTACTCTC
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GTGGAGTCTACAGAGGCAGGCAGGCCTCCTTGAGCTGCAGTGGGCTCCACCCAGTTCAAGCTTCCCGGCTGCTTTACCT
ACTCAAGCTTGGGCAATGGCGGGCGCCCTCCCCAGCTTGGCTGCCACCTTGCAAGTTGATCTCAGACTGCTGTGCTA
GCAATGAGCAAGGCTCCGTGGGCATAGGACCCTCCGAGCCAGGCACGGGATATAATCTCCTGGTGTGCCATTGCTAAG
ACTGTTGGAAAAGTGCAGTATTAGGGTGAGAGTGACCCGATTTTCCAGGTGCCGTCTGTCAACCCCTTTCTTTGACTAGG
AAAGGAATTCCCTGACCCCTTGTGCTTCCAGGTGAGGCGATGCGTCACCATTTCTTGGCTCAGCGTTGGTGTGCTGC
ACCCACTGTCTGCACCCACTGTCCGACACTCCCCAGTGAGATGAACCCGTTATGTGAGTTGGAAATGCAGAAATCACC
CGTCTTCTGCGTTGCTCAGCGTGGGCACTGTAGACTGGAGCTGTTCTATTTGGCCATCTTGGTTCCATCCCCCTACT
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CTTGGCTGCCCCAAGTGCTGGGATTACAGGCATCAGCCACCATGCCCTGGCTGCTAATAATAAATTTAAAAAACCTAAC
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CAAAATAAACAACAACTTTACAAAAGTGAATGGATTAGCAGGTTTGGGGCACATGCTTGTAGTTCCATCTCTCTGGAA
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CCCCTGGACCACAGAGGTGTAGCAGAGAATGGTTCTGTAAAGACTTGAAAAGTGAAGTACAACATAGGTGCAATTTGT
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GCTTAAACCTCAGACCCAATTAAATGGCAAAACATGACCCTACATCTGTTTTTTATGTATACACACACACACACAC
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GGAGTGCAGCGCACAATCTTGGCTCATTGCAACCTCCACTTCTGGGCTCAAGCAATTCTCTGCTCAGCCTCCCAA
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CCACTGCACCCAGCCAGATGTAGCATAATCTTAAAGGCCCTAGGATTTTTGGAATGGTAAAGGAGCACTGGTTTTCAAC
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AATGTAGCCACTTTTATTATGATCTTAGCTAGATTTTCTAGATAACTTGCTGCAGCTTCTACATTAACCTTGCTGCTT
CACCTTGCACCTTTTATGTTTGAAGACAGCCTTTTCTCAAACCTCATAAACCAGCCTCTGCTAGATTCCAGGTTTTT
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TTTATCATTTGTGTGCTCACTGGAGTGGCACTTTAGTCTCTTTCAAGAACTTTTCTTTGCATTACATAACTTGGCTGTTT
GGCACCAGAGGCTTAGCTTGTGACTTCTCTCAGCTTTTGACCTGCCACCCTTACTAAGGTCAATAGTTTCTTTTGATTT
AAGGTGACAGATGTGTGACTTCTCTTCACTTGAACACTTAGAGGCCATTGTAGGGTTATTAATGGCCCAATTTCAAT

Fig. 6.4

[illegible]

Fig. 6.5

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TTTTTTTTTTTTTTTTTGTAGAAATGGGTTCTCACTATGTTGCCAGGCTGGTCTCAAATTCCTGGCCTCAAGTGATCC
TCCCATCTCAGCATCTCAAAGTGCTGAGATATAGGCGTGAGCCACTGTGCCTGGCTAGAATATCCTAACTTTTAGCAA
AATCATACCTGAGATGAGGAAGGATGGCATATAACATGATTGAATCAGCACACTTAACTAAGAAAGATAGCCTAAGTGG
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GGAGTGATGACACGTTACGTTCTTGCTATTTTGGACTTATTAACAACGTGAGTGTGAGGGTTATGATAGTGTAGGCC
TGGCTGCTCCTGGTTCGGGAAAGAGTGCTATGAAAATTGAGATATATCTGTCTAGTGTATGGGAGGAGAAAATGGCTGT
ATTTGGTTCTATATATTTGTCATGCCTGGTTGATGGTTTGTATTTTCTATAGTGATTTGTGAGTGACAATGGATCTATA
TTCAATTGACCCAGTATAGTTGAATATGTGAAAATATTTATGTAATTTTACTAATATATATTTTGTAAAATACAACC
ATATAGAATCTACTAGGCTATAGGTGTTGAAAGATAACATTTTTTTTTTTCTAAATCAGGTTTGTACAGAAGGAGAAA
GTGGCAGTTGAAGCATTTTCAGATTTGCTGCCTTCTCTACCTCCTGAAAATAGGAGAAAGTTACAGCTATTGATGAGGA
TGATGGCAAGGATTTGCTTAAACAAGAGATGACCCCTGTGTGATGGCTTTGGTACCCGAACACTGGTAGGTTGATT
TTTAACATCAGGTATGACTTTTTGGAATGAAAGTCGACTGAGTGAAGGTGATTAGTTTGGGTGATCAGAGGGAAGTACA
AAGCAGACTTGCTTAAAGAGCCACAGTCAGTGTCCACCCAGACTTGTGTCTGCTTTTGTCTAAGTGTCTTAAAGAA
CATAATGAGAAATGGAGACTTGAGTAGGGGAGACTCTGTAGAGAGATCTGTACATCCTGTTCTCCCACTTAAATTA
TTTTAAATTTATCTTAATTGACAACATAAATTGCATATGTGATGTTTTAATTCATGTATACATTATAGAGTCAATCA
AGCTAATTAACAGATTCAATACCATACCAACTTATCATTTTTTTGTAATGAGATCATTAAAATCTATTCTTTTCTCAA
TTTTGAAATATACAATATATTATATAATTGTCGTCACCGTGCTGTGCACTAGAGCATTAAAGACTTGATCCTCCTGTCT
CACTGGATCTTTGTACCTTTTGACCAACATCTATTCACTGTCCTGTATCCCCAGCCTGTGGTACCTACCATCTAAT
CTCTGCTCTATTGGCATATGTTTTTAAAGAGAGTTTATTTTAGAATTTACTTACTAGCAAAATCTCTTGATTTTCCCT
AAAAATGTCTTTGAGCAAACTGGTGTCTCAGTGGAATGTATGTGCTTTCCATATCCTGTTTAAACACCGTGTACTCC
CTGGGGATTCACTGGCCAGTTTGTAGAAGTAATAGAGTGAGATCAGAGGAGCACTTAGTGCCAGTAGGATTCTTGATC
TCTATATTTTTTTGGAGTGAGTGAGTGATTAGAGAGGCTAATAGACCTTTTGTAAAAGCTGAAGAAAACTTTATATCTA
TTCTGCATATAAAATACACATGCAAACTTTGTCATGCAGTTTCAAGGCGATCACAGAGTCCCTGGGGTCCATGGTCTCC
AGGCAAAAATCTCTGCTGTGTGTCTCTCTGTACATGTAAATGTAAGTGAAGATCATTTGCAGTGTGTTAATGAAGT
GTTACTAAGCAGAATTACATGATTTTGTCTTAAAGATTTTGAAGGAAGATTGTGTCAGCAATAAAGTAGTAGGAAAAAA
CAAAACAAAAACAAAGCCAGTCCAAAGAACAGTATTAAAGAATGGAGTAGTTAGGCTGGGCGTGGTGGCTCACTCCTGTA
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ACCCCGTCTCTATTAAAATTACAAAATTAGCTGGGCATGGTGGCAGGCGCTGTAATCCCAGCTACTCGGGAGGCTGG
GGCAGGAGAATTGCTTGAACCTGGGAGGTGGAGGTTACAGTGAGCCAAGATCACACCATTGCCTCCAGCATAGGTTAC
AGAGCGAGACTCCGTAAAAAAGAGAGTCTCATCACTTAATGTACTTCTTGTAAAAGTCCCATGGCTTTG
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TGTGGACTGTGTTAGCGGTTTAAACAAAATCAATAATGAAATAGGATAAATAAATTGACTAAATGGCTATAAAGTG
CTTTGAACCTTAGTAGTGCTACATGATTCAAGTCATGGTGATAATGATTTTTTCAAATTTGTTTTTCATATTTATATGTG
TGATGTGTGTGTAT
ATTGCTCAGATAAGCATCTCTCTGCAGTCTCTTAACAGGAAGATGCACATTTTGTGTTGTTCTGGTTGTTGAGACCGTCA
ACCCATCTGAGATGGCTGGGTACTCTTGGTAGAATATGCAACCTGTGCCTTAGACCATTCTCATATTTGCTTTTGTCA
AATTAGATTGCACTCAGTTTCAGAAAATACTCATCTGCGTTTACCCCCACCCGCTCTTTTTTTAGATGGTTTCAGACA
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AGTCCAGGTAAAGGAGAGGATATTATCAGTTCTATGAAATTGGCAATATAAAGTACAGTAAGCTTGCTAGGCTTCTTGG
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GATTCATAATTGCCCTGTGACCTAGTTTAAATATTTCCCACTTTTTTGCATGAAAAAGCTAAGGCACAAAGAACCTTAAATAA
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ATTGCTGTCTAAAAATCATAGTACAGTAGAAGAACACTGGCATGAGAGCCATGAATCCCTGGCCAAATTCCTAGCTGT
ATCCTTTCTGTCTGTGACTCTGGGCAAGTCCCTTAAACCGCTTTGGATTTTACTTTCTTTCTCAGGCGTTAAAAATGGG
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GTATTCTTTGAAAGGTAGCTGCTGCTGTTGTGTCATGGTGGTTATATAAACAATGCAAAAGAAATATAATATATTATTA
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GACTCCTTCTGACTGATTGGACCTCTTCTCATTTGAGATCTTTTTGAGGCACCACAATCAAGGACCAGTGAAGTCCCTC
CTTCTGAGGTATTACAAAAATAATTAATTGTCCAGCTGTTCTAGAAGGCAATTTTAAAAATAAAAATTTTTCTATTTTT
TTTGCAATTATCAACCTAACACATTGAAGAAACTTTGGGAAATATAGAAAAAACACACCAAAAGATATCAGAGTTACACT
AAAATGTTATAGTCAAGTAATATAGTCTTTTCAAGATTTTTTTTCTGTGTCTGTCTGTCTGTCTGTCTGTCTGTCTGTCT
ACTTTCTTCCCTCTCTCTTCCCTCCCACTTACATTCTGTTAACAATGAGAAAAAATTCCTCCCATCATTAAT
ATTGTTGATTGGCATGAGAATGGCCAAGAGCACAGACTCAGGAGCCTTTGAATTCATGACATGCCTTTTGTAGCTGTG
TCACCTTAGGCAATTATATAACATGTCTGTGCTTCAACATCTGTCCCAACATTTCTTTTCTCATCTGTAATGGGA

Fig. 6.6

ATAATAGTAGTAAATTAATTTACCTCATAGAGTTTTTCTGGGGAATTAAACCATGTGATTAAATACATGTGGGCTTAATA
CATGTGAAATGCTCACAAATAATGTTTATCACATTGTAAACCTACAATTAGTAGCTGCGCTTTGTTGTTGGTATCATCATT
ATTGTTATATTATCCATAATAATATTATTGGCAACATAGTATTCCATAATATGGATATACCATAATTTATTTAAATGA
TACTTTTTGGTTGGTATTTAGATTGTTTTCATGCTTTTCCCCCATTTTGAAGCAATATAGCAGTAAATATTTTAAAGG
TAGATTTTTTTGGCTAATCTGTGATTATTTTTTAAAGATAAACCTCTAGGGGAGCAGATTGCTTGGCCAAATGGCCATGA
ACATATTTTTAAGTATCTATAGCATATTTGCCAAATTTTTAGAAATGATCATTTCATTTACATTTTCTGTCAGTGGTATAAGAG
AGTGTTCAATTTCTTTGCCCTTTGCCTACTTTGGATATTATCATTAAACACTTGTATATATCTTTGCCACTTGCATGGGT
GAAAAGTGAAATTTAACTGCTGTTTTAGTTTAAATTTTTTCTCTGTATTTTTTATGAGCCAACCTAAAGAAAATAAA
AATGAACAGAAATACCTTACCAAGTTTTCTGCAAGGAAATGTTATAGCAGTGTATTAGTCTGCTCTCATGCTGCTAATAA
AGACATACCCAAGACTGGGTAAATTTGTAAAGGAAAGAGGTTTCATTGACTCAGAGTTTCAAGATGAGATTGGGTGAGGAC
GGAAACTTACAATCATGCGCAAGGGGAAGCAACACATCTTCTCATGAGGCAGGAGAGAGAAGCACCGGAGCAAAAC
GGAGAAAGACCCCTCATAAAACCATCAGATCTCATAGAACTCATTACTATAGAAGACAGCATGGGGGGAACCACT
CTGTGATTTCAGTTTACCTCCCATCTCCCAAGCAAGGGGATTATGGGAACACATTTCAAGATGAGATTGGGTGAGGAC
ACAGCCAAACAATATCAAGCAATAACTGTGTTGCCCTGTATACTTGTCTAGGTTTGTGTATTACATGCATAGCACAGGC
ATATATTGTAGTAACCTTAGCCTTGTGAGCCCTTTGCTATTACTTGAAGTTTCAAGAGCTGAGCTATGGTGATTAAATTA
ACTGCAAGGTAAACATGATCTTGTTAAGAGACACTGCAGTGTGCTCTGAATAAAATCAGTAGTGATTCAATTTGTCCAGTT
ACCTTTTTCTCTCTTGCAAGTACATATTAGAATTGCCAAGCACCTTGTCCATTTCAGCCCTCAGAACCTATAGATCTTTGT
TCTTTTGATTAGGCTCCTAAGCCCTCTACACTGTATCATTATAGGGGAGTGCTTCTCTGAAATGAGCTTGTGCTTGGGA
TATTCTCAGTGAATGTTTTTAGGACAGTTTTCAGGGCTGACCCCTGGGATAGCTATTATAGGTAGGCAATGTTTTTGACACA
GCCCTTCTATGATCCTCATCTGCAACCTTCTATTTCAAAGGACTCCAGGCTCTGAAATTAATATTTTTTAATGTATGTTT
ATTTATTCACAGGCTTGGTTTTCTTTTTCTCTTATTTCTCTCTTCTCCCTTATCCCTTCTGCAATGTCTGCACCTTT
GTTGTTTTACTTGATTCTCATGGGTAAGATGTTAGGAAATGATTGTAGTACCCCTCTCTTCTTAAAGCTTAGCTAAAT
GCNTGCCACTACCATCCCCAAGGCATTGAGAATCACACTCTTCAGATGTGGGAATGCGCCTGATAGTTCCAGTGGATA
TCCAACATTATCAATGTTTTGAATCANTTTAGTCAATGTGTTTAAATTTATGCTTGAATTCAGATCTTGTAAAAAGAGG
GAATGCCGAGACAGCTCGGTCTGGGAGACCTTAACCCAGTGGCCCTTAGAGGAATTAAGACACACACACACACACA
CAATATAGAATTGTGGAGTGGGAAATCAGGGGTCTTACAGCCTTCAGAGCTGAGAGCTTTGAACAGAGATTTACCCACA
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AATGCCTTAAGCGGTTTTTCGCGCCTGGGTGGGCCAGGTGTTCTTGCCCTCATTCCTGTAAACGGACAACCTTCCAGCA
TGGGCATCAAGGCCATCACGAGCATGTCACAGTGTCTCAGAGATTNNGTTTATGGCCAGTTTTTGGGGCCTGTTCCCAAC
GAGGGCACAAAGCTTTTTTTGTTATTGAAATGGCTCTTGGGTTTACAAAGGTAAAAATCCAATTTAAACCCGTGGCTTTA
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GAAGTCATAACAGATGCCAAACTCTCCAACAAAGAGAAAAAGAAAGTGAAGCAGGTAAAGGATATTCTTTTATAAG
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ATAGCAGTTTCTTCAAGTATATTAGTTCAATATCTAGAAGTCTCACTGATTTACACACTTGTGGTCAGTATCCTAGAT
CTTTATTCTTCAGTGTAATCTGTTTACCATTGCCAACTCTTCATTTTGGAGCTATTCTCTAATATTGTTCTTTGTT
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CATGTTTTCAAAGGCAAAATCAGATGGAATTCACAGGTGTAAATGTTTATAGAGTTTAAAGCCGTTGGTTTTAGATAGC
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CAGAAAGTTGCAGCACTTCTGTTTTCTGTAACCAACCAAGCAAGCTGCTAATGTGGGCACATAAGAACGCTTTT
CCAACCAATTTCAAAGAACTAGAAGTTTTGCAATGTAAATAACTTCCACAGCAACAGGTGCTAGAGACCAGCTTGTGTTG
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GATAAAGATTGCCCTAGTTTTTAAAAATGTTTGGCCATTAGTATTTTTTATAAACTCAATGCTAGTTTTTAAAGTGTATA
AATTGGTTAAATTTTATGAGTCAAATATATAGTGATAATGTTAACATGTTTGAATTTGCTACAGAATTTAAGGGTATTT
TTATCTCTGTGCTTTCTTTTTTTCATGGTGTTTATTAATAATTTGTGTATATACATCCTAGCTACTGATATCTTTATTATA
GCCTTAAGACTTAATTTTAAAGTCTTAAAAATACGCGTGTATATCTGAAATAAGAAAGACACTGGGTACTGTTACTGTGATG
CTATTGACTTAGTAGCCAATATCATATTTCTCTGTATAAAATCCAGTTTATTGCTGCACATAAAATTTTAAATGTCT
TATATTGTGATAGCTATGTCTTTTTTATGAGATTTATGGATGTTTATGACAGATTTTACTAAAGCTAGTGTTTTTATAA

Fig. 6.7

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CATATATATTAGTTGATGTTTACCTATAAGTGGAGTAGATTTTCATCTGCCTGCAATGGTATAATTTTCAGTCTTAGCTA
AAAATGGAAAGTTGAACTGGATAAAATCTTTGGGTACCTTAGACCTCTGATTCTAAGTCAAATGCAAATGGGTAAAT
AAAATGAGACTACTTCTTTTATAAATATATTTTCATCTTTTGAAAGTAAGTGAAATGTAAATAAATTTATTTTTTTA
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CTTTGCCAATTTTGTAGCATTGCTTTGTCTTTTGTAAAATTGTGGTGCACCTGGATTATGGAGCATCTGAAAGTCTTC
CTCTGAGCGCAATTACCCAGAAAGCTGAGAGCAACACCCACTAATTGGCTTACAGTACTCTCTGGACTGATTTTTCTT
GCACTTTAAGATTTGCTCCTGTCTCTTGTAGCAAAATACCTCCTACATCTGATGCAGATTTTGTCTTTAAAAATGGACC
AAAGTATTCTTATTTGGTTTGGGTACACCTTACATTCCAAATACATAATGTAATGGGAGATTTTTAGTGTTTCAGGATT
CATTCTCAGACTTTGGCATTCTGTTTAGAGCCAAGAATAATTCTTCTTCTGCTCTATTTTCACTCCGAGGTAGAGTTT
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TTGATTGTATTATATGTATAAATCAATTTGCAGAGAAATGCTGTCTACAATATTAGGTCTTCTAGTCCATGACCATGGT
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TGATTTCTTTGGCATTGTCTACNTATTAGGAAAAAACAGCTTTTGTCTTTCCGTTCCATGGTAGACAGCATCTATATTG
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ACATTTCTAAATAATAGAATATATCATCAGTGATGGGTATCATTCTCAGATTAGATTCCAAACTCTGGCTTCCATC
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CTTGAAGTGGATCTTCCCTTGGTCAAACCTTGAGGTGACTTACCCTAGCTGACACCTTGATTGGAGACTTTTGAGAA
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CACTTTCTCTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT
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AAGCCACGCTCAACCACAGTCAGAAAATATTAAATGGAAATATTCAGAAATAACAAGTTTAAATNGTGTGTCTGTT
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AGTCTAGATGACATCTTCTGCCAATAGAACGCTGTTTTATCTACAGTGAAAAGCTATTGTTTAGTGCAACCACCTTCA
TCATTGATCTTAGCTAGATTTTCTGGATAACTTGTCTGAGCTTCTCCAGCCTTTGTTGCTTTCACCTTGCACTTTTCAGT
TAATGGAGATGGCTTCTTTCTTTAAACCTCATGAACAGGCTTTGCTTGTCTTTCAACTTTTCTTCTGAGTTTCTCTAC
CTCTCTCAGTCTTTATAGAATTGAGGAGTTAGGGCTTGTCTTATAGTAGGCTTTGGCTTAAAGGGAATATTGTCTGCTG
TTTTATCTTCTGTCTGACCACTCAAATGTTCTCCATTTTCAGCAATAAGGCTGCTTAGCTTTTGTATCGTGTGTGTTTA
CTGGAGTGGTGTCTTTAATTTCTTTCAAGAATATTCTTTTCACTCACAACCTTGACTAACTGTTTGGCACAAGAGACC
TAACTTTCTTTCTATCTTGGCTTTCAACATACCTTTCTCATTAAGCTCAATCATTATAGCTTTTCGATTTAAAGTGAG
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TGTAATAATATGCACTGATCTGGATACAATATACAATATAATCTGTGAAGTGCAATGAAACCAGATATGCCTGTGTATAT
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GGAATAGAGTGGGGATCGCAGCAGCATCATCTGCTCCTAATTTCAAAGAGGAGGTTTTTAGCATTAGAGTATTTGTAG
ATACCTTTTATAATTTTAAAGAAGTTATTTTCTATTCTATTCTATTGTCAAAAAATTTTTATTGCTCGTTTTAACC
ATAAGTTGATGTTGAATCTTAATCAACTACCTTTCTTACATTTGAGGATTTTATAAGTTCTATCTTTTAGTCTATCATT
GGGTTATATTACATTAATTGATTACTAATATTAAGCCACTTTGCATTCTAGGAGTGGCATAAATCTAATTATGATGTA

Fig. 6.8

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TTATCATTTGAATATATATATCTAGATTCTGTTGCTAATATTTAGTTTATAGTTCTTGTATCTATGTTTCATGAGTAAAA
TTGGCCTGAAAAATTCCTTCTCTTACTATCTTTGTTGACTTTCTGATCAAGGTTATAAATTAAGCTGAAAAATTAAGCTCTC
TTTTCTATATATGTTTGAATATTTCTTTCTTGAATCTTGGGTGAACTTAGTTAACTTTAAAAAATAAATAATGT
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AAGTAGCTAGGACTAATTATGCTCAGCTAATTTTATCAAATTTTTTATAGATTTTGTCTTGTCTGTGCTTGTACCCAG
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ATTTCTGATTAATTCATTTTGAATTTTCAATTTATCTGGTGGACTGACCTTTTATTTATCATTATAAAATGTCATT
CTTTATCATTAGTAATGTGTTTGTCTGTAGTCTTTCTGTATTATATTTAGGTGTATCTCTTATAAACAGCATGCTTT
TATGTTTTTAAGAGCCAGCTTTCACAGTCTTTATTTTATGATTTAGTATTTAGACAGTTTATATTTAATACGATTACTCT
AATTTTGGGTATAAATCTACTCTAATTTTGGGTAAAGCACCTTTACTGATGCTTCTAACATTATCTTCACTTTCTTG
CCTTTGTTTGGATTTTGGAAATAATTTATGTTTCTCCTGCTAATTTTGAAGCTTATGCACTGTTTCCAGTCTTCTTG
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TTATTTCTTTTAAACAGCTTTTATGAGATATAAGTCACATAACCATAACAATTCACCCACTTACAATGTATAATTCAGTGA
TTTGGGGTATGTTACTTTTTTTTAAAGTTGTGGTAAAATATATATGGCAGAAAATTTGTGTTTATGCCATTCAAGTGAC
AATTTAGTGTAAGAATGCCATCATTTGTTTAAAGATAGTTTAACTGTAAGTTATGGACTAAATATTTGTCTCCCAGAA
AACTCATGTGTTGAAATCCTAACCCCAAAGTGATGGTATTAGGAGATGAGGCCTTTGGGAGGTGATTAGGCCAAGAGA
GTAGACCATGAATAGAATGAGTGCCCTTATAAAAGAGAACCCACAGAGCTCTCTTGCCCTCTTTCTATCGTGTGGGGAT
ACAATAAGAAGTCAGTAGTCTACAACATGGAAGAGAGCCCTCACCAGAAATGGACCATGCTCACACCTTGATCTTGGAC
TTCCAGCCTCCAGGATTATGAGAAATAAATTTCTATTATAAGCCACCCAGTGTATGGTACTTTGTTATAGGCTTGAA
CTGACTAATACACTGTGTATAGAATTCTAGGTTGATAGCTATTTTATTTCCATATACTGCAGTTATGCCTTTGTTAGCT
GGCTTCCATTACTGCTGTTTAAAGTCAGCTGCCACTCTAGATATCACTTCTTTATCAATAATGTGCCCTTTCTTGGC
AGGCTGCTTTTCTCTCTGCTTTCTGTTGCTTTTCTGTTGCTTTTCTGTTGCTTTTCTGTTGCTTTTCTGTTGCTTTT
TGCTTGGGATTTCTTGAGCTTCTTGAATCAGAAGTTGAGGCTTTTGTGCTGTTTAAATATTGCTTTTGTACAATCTC
TCCTCTGCTTTTGAATCTTTAATTAATATATGTTAGCTCTCACTCTGCTCTTAATCTCAATGTATGCTGTATTTTN
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TTGTCAATTTTCAACCTATACCTTAAAAAATTTAAGATCTCAAATTTCTTTTGTGATTGTCAGCATTTCATGTCTTAA
CCAGCTTTGCCAAATTCATTTCCAATTTCTTTTGTCTCTTTCTACAAATATATGCTAAAGATTTTCTATCGAATTTTC
CACAAATTTCCACAAAAGATACTGCAACAGTGATGAAAACAAAGTCTTTCTTTGATGCTCTATAGACATAAGCATGTAT
TTGTTTTATTTTAGGTATGATTTTATAGTATTTTCAATTTTATGTATAATGCTTTTATTTTATGATGATAAGT
CAAAAATGTATAGGGAGTATCATTTGCCCTAAGAGACTCTGGTAAAGAAATTTATCAAAATGAATGTTCCATAGCAAAAT
TTTGAAGAGTACTACAGCAAAATTTTCAAGGTTGAAGACACTCTGTATGAGCAGGGTGATGGAATTCAGCCATTTCTCA
TTTTATTTCTCACACGCTTTTGTGTTCTGCTCATCTGCTTTCAGGGAAGTCTGTCTGCTGGGAGTCTAGCAAAATTAATAG
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TGTTATAGGAAATATAACTTTTAAAAAGCTGATGTTTGTGAGCTGCTGTGTACAGGAAGAAGAACTAAAAATGTCTT
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GCTGTGGTAAATCATTACTTTTACCCTCAAGAACAAACCCCTATATATGTCAAAGACCTAGGGAAGTAAAGAGTTTAA
TTACCTATCAAAAATCAGTATTTAGGAGAGATTCACTGTATTTATATTCATCATCATAGTATTTTGAAGTACTCACTT
CAGCATGAAACATAGGAAATTTCAATTTTACAGCATTGCGATCGTGCGTTTTCTTGCTTAAAGACAATATAACCTGCAG
AGTGAATACCTTGACATCACTGGGTCTTCCAACAAGTTGCCGTAAAACATAAACTATGATTATTGAGTCTTAAAGAA
ATTATTTGCTCATGGGTACTCAAGTGATTTGAAAGTTGGGATCTAAGACCAGTTAATGAACAGAAATTTGCTACTTTGT
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CCCTCTGATGAATTTTTTCAAGTGTCTAGTTAACTTAACTTTTCAATTTGAAGAAATAAACTGGGAACAGTAA
TGGGACAACAGGGTGCTAACCAATAAAAAAGTCACTTTTCAAGTTTGTGTAGTGCATTTATGTTGCAATGTAGTTTCT
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GTTTGTCTTCAATTTGAGGATGTAGAATAAATATATTTTCCATAAAATGTACCAACAGTGCTTATTTCCAAACAAAAA
TAATTATGTATGTGGCTAGGTCTACTCACTTTGTAATACCTCTTTTGGGGCTCTTCTGTGCAAGCTGAGGACACTTACA
GTATCTAGAGCCTTTTCTAGGGAATATAACAAAAGCTTTTCTCCCTCTTCTAGCCCCACAAATTTTACATCTTGTCTGC
CAGCGTATCTTTCAAAATAATAACAAGGTAGTCTTCTTGAGTAGTTTATAATGGATTCTTTCTATGTTTAAATAAGCA
AATGAACTTAATTTTTTAAAAAACTAGAGGACTAAAAATTTTTCATGAGAACTAAGTAAAAATATAATTGATATTTG
TATTAGTCCATTCTCATGGTACTATGAAGAAATACCTGAGACTGGGAGATTCTAAAGAGAAGAGGTTTAACTGACTCA

Fig. 6.9

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CAGTTCGTCATGGCTGGGGAGGCCCTTAGGAACTTACAATCATGGTGGGAAGGTGAAGCAAACACATCCTTTTTTCACAAG
GCGACAGGAGAAGAAGCGCCGAGCAAAGGGGGAAAAGCCCCCTTATATAACCATCAGATCTTGTGAGAAGCCACTATCAT
GAGAACAGCATGGGGATAACTGCCCCCATGATTTGATTACCTCCCCTGGGCTCCTCTCAGCACAGTGGGAATTATGG
GAACTACAACCTCAAGATGAGATTTGGGTGGGGACAGCCAACCACATCAATATTTAAATTTGAAGTACTTCAGTTTTG
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GGTCTGACAAACAGACATTTTCAGTGATAATTTACCAAGATAGCAGAACTGTTTGTGCTAAAAATAGTTATGCGAGGATA
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CTGATTTAGCATATTTGACTATTTTACAAGTCACAATCCATCTAACAAGGACAGATGTTCCCAGTAGAGGTCAGTCTT
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GCGGACTTATTTTTAAGTAAGGAGTTTTTTGGCACCTGTCAAATAACATCTCCGAGATGGCTTCCCTTTACATATTCCAG
GTCAGTTGATCCAGATCTCAATGCTCTTTGCAATTTTTGGATTCTTAGTAGGTTGCTGCTACACCATGGTTTTCTCTTTC
CACATTTTACAGGGCATTCTGCAATTTAGTCTGTACTGATGAGTTTACAATGGTTGCAATAATTTGTTGGTGTGATGAA
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GTTGCTGTCAGTGAGATGCTTTTAAATTTTCTCCACATGTGAACCTCAGTGTTTTCTTTTAGGTTTTCTTTAATTTCCA
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CTCTCAGAAAATATCTTTTCAAGTAGCTCTGTGTGCTCAGACCCCTGGAACCAAGTCATTCTGCTGCAACAGAGGCTG
TGCTTTTCTATTGCCACCCATTTTCACTGCTCAGTGGCTCTTTCAGATATTAGTGCTATTTCCATAATTCTAGTTGCAT
ATGCTTTTTTGTCTTCTTACCTATGCCACTTAAATATTGTGACATTTTGGGAGCACTTAAACATTTACTTCAAACTTCA

Fig. 6.10

[illegible]

Fig. 6.11

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AGTATAAAAAATCTGTTGTATAAAATGAGGCTAAGACAACCTAAGAAATTGAGGATATTTTGTAAAAATCTGGATGGAT
 TCTGCTCCCAAGTAACCTTTGCAAGTGACATGAACCTTTTTTATTCCACTTTAAAGAAATTAATTGGTGATGCAAAAAGT
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 GTCTACAATACCACATCATAAGCTATTATCTACAAAAATGGTAATAAGAGCATAGGTATGAGGTTTTAATAAATAATAT
 TTTACAACAATCAGTAATATTTTGCAGCAATGCAACTAGAGTAAAAAATTTGCTGTTATTGGCAAATTGACAAAAGAGGTT
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 TGTAGGCTGGGAATGACTCAACAGTTGGGGGTCTGGAATCATCTGGTGAATCTACATTCATTGTATTGCAGTTGATT
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 GACACACGTAGGCTCAAAATAAAGGGATGGAGGAGACTATACCAAGCAAAATGGAAAGCAAAAAAAGCAAGAGTTGCA
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 CTGAGTGACCTACAAAGAGACTTAGACTCCACACAATAAATGGGAGACTTTAACACCCCACTGTCAACATTAGACA
 GATCCATGAGACAGAAAATTAACAAGGATGTCCAGGAATTGAAGTCAAGTCTGCACCAAGCGGACCCAAATAGACATCTA

Fig. 6.12

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CAGAACTCTCCACCCCAAATCAAAGAATATACATTTTTTTTTTTCAGCACCACACCACACCTATTCCAAAATTGACCAC
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GGGAAATGTGGGGTTGGAGCCCCACACAGAGTCCCTCCTGGGGCACTGCCTAATGGAGCTGTGAGAACGGGGCCACCA

Fig. 6.13

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TACTTCAGACCTCAGAATGGCAGATCCACTGCAGCTTGCTCTGTGTGCCTGGAAAAGCTGTAGGCACCTCAACACCAGC
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GCAATCTCATTACTGGGTATATACCCAAAGGATTATAATCATTCTACTATAAAGACACATGCATACGTATGTTATTG
CAACACTATTTACAATAGCAGAGACATGGAACCAACCTAAATGCCATCAATGATAGACTGGATAAAGAAATGTGGTA
CACATACCCATGGAAATCATATGCAGCCATAAAAAATGAATGAGATCATGTCTTTGCAGGGACATGGATGAAGCTAGAA
GGCATAATCCTCACCAACTAACACAGGAACAGAAAACCAAAATATCGCATGTTCTCACTCATAAGTGGGAGTTGAACAA
TGAGAACACATGGACACAGAGAGCGGAAAAACACACATCCAGGCCTGTTGGGATGTGGGGGGTGGAGGGAGGGAACCTTA
GAGGATAGGTCAATAGGTGCAGCAAAACCACCATGGCAGATGTGTACCTATGTAAGGAACCTGGACATTCTGCACATGTA
TCCCATTTTTTAGAAGAAATCAACAAACAAACAAAAAACTCATTTCCTTGTAATTTGCTCAGTCTCAGGTAGTTCTTT
ATAGTAAATGTAAGAAAAGACTAATCATCAAGGTCACTATTCACAGTGCAGTACTGGAGTGAAATGTGTTAAGAAAAAGA
TTTCCTAAACTTGGAAATAGCCATATGGAGAAATGTGCAACAGATCCATCATTGATGATTTAATTATTTAAGGCTGTT
AACTTTGATTTAACGCTATTGATTGGGATATTTACAACTCATGATAGATGGTGACTTATGATAAGTGGAGTGGTGC
AAATAAATTTCTCCCAGTAGAAAAGAACTCCAGGGGATATAGTTAATGCAAGACATTAACCAGTTTTATATCTCTTT
TAGCATTTTTTATTAGTCACTCTTTTTCAATAACTATAAATACTTCTGCTTCTGCTGTATTCTTTTTCTCACCTTTT
CTATTTTATTGTTTCCCATAATAATGAGTTAAATAAATCTTTGATACATGCTTACTTTATTTTATATGAGAGTCAGGTT
TTTAAATTTATACTTTGACAAATGAAAGTCTCAGTGAACATGTACTACATCTTGAAAAAGGGATGGCTGGGGCTGA
TAATACTATAAAACAAGAGTTGACAGACTATGGCCCCATGGGTCAAATGCACCTTGTAAGAACGTTTTATTGGAACATAG
ACACACTCATTGATATGTCTTGTCTATGGGTTTTGTTCTGCTATAGCAGAATTGAGTAGTTGCAACTATGATCATATG
GCCATAAGGCCATAAATATTTTATCTGGCCTTTTACAGAAAAATTTTGCTAAATCTGTTTAACTAGGAAATCCTGAAT
TTTAAGACTAATCATGAACATAATTTGTTAATCGTTCTCAGAAAAATTGACAGCACTAATGATTTATAGACATCAAACA
ATAGAATATTTAATTTGATTTACTAGAAAATTAAGAATAATGCAGAATAATATATACTCTGAATTAACCTTATTGATCT
CAATTTGGAAAACAGAAAACAATGAAGAGATATAATCAAATCTAGAGCCAAAAGTGCCCAATAACTACCTAAATATTAC
CATATATTAACATCAGATGGAATCAACTGTCAAGAATATAATTAAGAGGTGGAGGACTATGCTGTGAAAAAATCT
ATCATCTTAACAACCTGAACTCATTTTTTATTAATGTATATACATTTAGTTTAAATGAAAATATAGTATTTGTGTTATA
TACTTTTATTCTCACTTTGACTTTTTAATTAATGCTCTTGATTTTCATCAGATAAGAAGGCATCTGGTGTATTAAGTT
TTGTTAATTGAAAAATCTCAACTTTTTTATTGCCCCAGCAGGGTAGAGGCTTGCTCAGAAGCTTGGACTAGCAACATAA
CTAGCTTTTTTTTTTTTTTTTTTGTCTTCTCTCTCTCTCTCTGCCCCTTAATGTAGGGTGAATGAATTTGCCAC
CTCAAAGCTATCTACAATGGGTGAGGCAAAATAACCACAATATGGGTTTGTACATAATTTGGGAAGGAGTTCTTCTCCT
GTCCCTTCTCTCTTTGGCAATGCCTCTTAATCACAGTTGTGTTGGTTGAACAGCTCTATGAATATATGAAAAGCCACT
GAATTGTGCACTTAATTGGGTGAGTTGCATGGTATGTAAAATACATCTTAATAAGCTGACACCCCAAGAAAATCACAG
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TGCAATGGCCTGATCATGGCCTATTGCAGCCTTTGCCAAAGGAGCTCAAGGGCTCTCCACCCAGCCTCGGAGTAG
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TGTCCAGGCTGGCCTTGCACTCCTTGACTCAAGCCATCTGCCCATCTCAGCCTCTCAATGTGCTGGGGTTAGAGGGATG
AGCCACCATGTCTAGCATAGAGTTGTATTTCTAGGTTTAAAGTAATTAATTTATGGATCTGCAAGCCCAATAATTTT
TATAAGTTATAATTTTTCTTATTAATTAATAAGTATTTCCAGGGACTCTGTGAAGTCTGTTATGTCTGGGCTCACGTA
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AGATGCACTCATCCAGGTGGGAGTGTTCGTGCAAGCCAGCTTATGTGCCATGCACCAGATGTCAGTGGATGGGGGTG
TGTGAATCTGTTAGGTAGCAGAGCAGTTTGGCCACCAAAATATACACAATGTAGCAATTTCCCAACAATATATTTAC
AAATCTTTTACATTAAGCATTACTGACATTATAGAAGTGTCTTTGGATTCTTTTATTAGTTAAGGTGAGCATATATTAC
GTTTGTAAATTTAAGTCGAGTACATAATAATTAATAAGAGAGATTTTTTATTCTTTCAAGTTTTCAGGTACAGAGATAGT
CTCTTTGCTTCGATATAGGGTACACTAGAGAGGGTTGTACACCTCAGTTTATGCTCCACACTCTCCAGCAGGGAGCA
TAGAACCTTGACCTTTAGAACATTCCACTTAATGGTGAATAACCTGTATGCAGTGAAGCTGGTAGGAGAAATCAAGT
AAGAAGAGGAAAGAAATGGCCATAAAATGCCACACATTTCTCTTCGCCCCCACCAGCCCCCTCAATCATAGTGATG

Fig. 6.14

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CTCCATTGGGCACTCTTTACCTCAACTTAAGTTTCACCTTTTTCTGGTTTGGAAAGGGAAAACACTACGTTTTTTTGT
GAGACGGAGTCTTGCTCTGTCAACCCAGGCTGGAGTGCAGTGGTGCATCTCGGCTCACTGCATCTCCCTCCCAGGTT
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TTAGTAGAGACGGGATTTCACCATGTTGGCCAGGATGGTCACGAACTCCTGACCTAAGGTGATCTGCCCTCCTTGGCCT
CCCAAAGTACTGGGATTACAGGCGTGAGCCACCGCGCCCATCCAACTACATTTTTAAAAAAGTTAAAAAGCAAAAC
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GGACCATTGTTTACAAGGTTGAGAACTGTGAGTGTACGGAACCCAGGGGAAACATTTTTCTCATTCTCAAAAT
AGAGGTAGGAATAAGGCATGGAAAAATATAAATGAGTAAATATAATCCTTGTACCTTGAATAAATTGGTGATAAAGTG
CAGGTGCATCTTATTTTCATTCTATCAAGTCGTTTTCTAATTGGATGTTCTTGAAACAGTGGCTCATTGCTTGACTTG
TTCACCTCCTATTTCTTGCTACTATCCTGTTTTGGGGGAGGGCAACTCTACCCTGGGGAACGATCAAATGTTTAGGCA
AATATATCCTGGTGTTATAAGCTCATTAAATTTCTTGTCATTTCTTGCACTGTGCTGGGAAGTGCTGTTTGTGGCCGT
TTTTGCAATGTAGAACCTCTCTTTTGTGAGAGCCATGGAAGTTCCTTATTTGATAATTAGTCTCTTGCTTTAGGCAGTG
CCATTAAGAACTCCATATAAGCTGCCAACATTTGTGTGATGATGTTCTTGTGTTAATATAAAGCTTCAGACTGGAGGATCA
GTAAATTTTCAGATGCTTCTGAAGTCTTACCCACTTATCTTGGCGTAGAGGTTCTCAAGCTTCAGACTGGAGGATCA
CCTGGAATTGCTCGTTAAGACACACATTGCTGGGATCCACCTCCAGGGTGTGAGAGTCAAGTCTGGGGTGAAGCCTGAG
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TGGATTAAAGAGCTGACCTGCGATCTACAGCTCAAATAGTGAAGTAAACATCCTAAAGAAAATGGAAAAACAGTGCAGT
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TGCTTATATTTGGGTCCTGCCTTCTATAAATGCTCAGATTTGCTTTTATTAAGATCATACACTCAGTGACCTGAGGA
CCAGATGGAGGTTATAAGCAGCTCTTTAAGGCTTCAGAGCTTAGCCTAGAGAGTCAAACAGCTCTTTGAACTGGCGTCT
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GGCTTGGTTTGAAGTCTTTGCTATTGTGAATAGTGTGCAATAAACATATGTGTGCATGTGCTTTATAGCAGCATGAT
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GGTTTGTCAAAGATCAGAAAGTTGTAGATATGCAGCATTATTTCTGAGGGCTGTTCTGTTCCATTGGTCTATATCTC
TGTTTTGGTACCAATACCATGCTATTTTGGTTACTGTAGCCTTGTAGTATAGTTTGAAGTCAGGTAGCATGATGCCCTC
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TTGATTTTTGTATCCTGAGACTTTTCTGAAGTTGTTTATGAGCTTAAAGAGATTTTGGTCTGAGACGATGGGTTTTCT
AGATATACAATCATGTCTGCTAAGCAGGACAATTTGATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
CCTGCCTGATTGCCATGGCTAGAACTTCAACACTATGTTGAATAGGAGTGGTGAAGAGGGCATCCCTCTGTCTTGTG
CCAGTTTTCAAAGGAATGCTTCCAGTTTTTGTCCATTGGTATGATATTGGCTGTGGGTTTGTATACATAGCTCTTA
TTATTTTGTAGATACGTCCTCAATACCTAATTTATTGAGAGTTTTTAGCATGAAGGGTGTGTAATTCTGTCAAAGGC
CTTTTCTGCATCTATTGAGATAATCATGTGGTTTTTGTCTTTGATTCTGTTTATATGCTGGATTACGTTTATTGATTTT
CATATGTTGAACCAGCCTTGATCCAGGGATGAAGCCCACTTGATCATGGTGGATAAGCTTTTTGATGTGCTGCTTGA

Fig. 6.15

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TTCGGTTTGCCAGCATTATTATTGAGGATTTTGCATCAGTGTTTCATCAAGGATATTGGTGTAAAATTCTCTTTTTTTTGT
TGTGTCTCTGCCAGGCTTTGGTATCAGGATGATGCTGGCCTCATAAAATGAGTTAGGGAGGATGCCCTCTTTTTCTATT
GATTGGAATATTTTCAAGGAATGGTACCAGCTCCTCCTTGTACCTCTGGTAGAATTCGGCTGTGAATGCGTCTGGTC
CTGGACTTTTTTGGTTGGTAAGCTATTATTATTCCTCAATATCAGAGTCTGTTTTTGGTCTTTTTCAGAGATTCAACT
TCTTCCTGATTTAGTCTTGGGAGGGTGTATGTGTCCAGGAATTTATCCATTTTTTCTAGATTTTCTAGTTTATTGTG
TAGAGGTGTTTATAGTATTCTCTGATGGTAGTTTGTATTTATGTGGGATCGGTGGTGATATCCCCTTTGTCAATTTTTTA
TTGCATCTATTGATTCTTCTCTCTTTCTTCTTTTATTAGTCTTGTAGCGGTCTATCAATTTGTTGATCTTTTCAAAA
AACCAGCCTCTGGATTCAATTGATTTTTTGAAGGGTTTTTGTGTGCTATTTCTTCAGTTCTGCTCTGATCTTAGTTA
TTCTTGCCTTCTGCTAGCTTTTTGAATGTGTTGCTCTTGTCTCTAGTTCTTTAATTGTGATGTTAGGGTGTCAAT
TTTAGATCTTCTCTGCTTTCTCTTGTGGGCATTTAGTGTCTATAAAATTTCCCTCTACACACTGCTTTGAATGTGTCAAG
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AGTAGTCATTAGGAGCAGGTTTGTTCAGTTTCCATGTAGTTGAGCGGTTTTGGGTGAGTTTCTTAATCCTGAGTTCTAG
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ACTATGTGGTCAATTTTGAATAGGTGTGATGTGGTCTGAGAAGAATGTATATTCTTTTGAATTTGGGTGGAGAGTTT
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TCTGTCTAATGTTGACAGTGGGGTGTAAAGTCTCCCATTTATTATTGTGTGGGAGTCTTAGTCTGTTTGTAGGTCTCTA
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ATCCCTTTTACCATTATGTAATGGCCTTCTTTGTCTCTTTTGGTCTTTTGTGGTTTTAAAGTCTGTTTTATCAGAGACTAG
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CTCTGCACATGAGATGGGTCTCCTGAATACAGCACACTTGGTAGGTTGACTCTTTTTTCAATTTGCCAGTCTGTGTCT
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CCTGGTTATTTTGTCTGTTAGTTGATGCAGTTTCTTCTAGCCTTGATGGTCTTTACAATTTGGCATGTTTTTGCAGTG
GCTGGTACCGGTGTTTCTTTTCCATGTTTAGTGCTTCCCTCAGGAGCTCTTTTAGGGCAGGACTGGTGGTGACAAAATC
TCTCAGCATTTGCTTATATGTAAGTATTTTATTTCTCCTTCACTTATGAAGCTTAGTTTGGCTGGATATGAATTTCTG
GGTTGAAAATCCTTTTCTTTAAGAAATGTTCAATATTGGCCCCCACTCTCTTCTGGGTTGTAGAGTTTCTGCAGAGATAT
CCGCTATTAGTGTGATGGGCTTCCCTTTGTGGGTAAACCGATGTTTGTCTCTGGCTACCCCTAACATTTTTTCTTTCTAT
TTCAACTTTTGGTGAATCTGACAACTATGTGTCTTGGAGTGTCTTCTCGAGGAGTATCTTTGTGGCATTCTCTGTATT
TCCTGAATTTGAATGTTGGCCTGCCTTGCTAGATTGGGGAAGTTCTCTGGATAATATTCTGCAGAGTGCTTTTCCAAT
TGTTTCCATTCTCCCCGTCACTTTTCCAGGTACACCAATGAGACGTAGATTGTTGTTCTTTTACATAGTCTCATATTTCTTG
GAGGCTTTTGTTCATTTCTTTTACTCTTTTTTCTTAACTTCTCAATTCATTTCACTTTTCTTCCATTACTGA
TACCTTTTCTTCCAGTTGATCGAATCGGCTACTGAAGCTTGTGGATGCATCACTTAGTTCTCGTCCATGTTTTTCCAGC
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GTACAGATGGGGTTTTTGTGTGGATGCTCTTTCTGTTTGTAGTTTCTTTTAAACAGTGAGGACCTCAGCGGCAGG
TCTGTTGGAGTTTGTGTTGGAGGTCCACTCTGGACCTGTTTGCCTGATATTACCAGCAGAGGCTGCAGAACAGCGAATAT
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TGGCCATGTGAGGTGTGCTGCTCCTACTTGGGGGTGCTCCTCAGTTAGGCTACTTGGGTGTGAGGGACCCACTTGA
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GGTGGGGCGCCCTCCCCCAACCTTGCTGTGCTTGCAGTTTCAGATCTCAGACTGCTGTGCTAGCAATGAGCGAGGCTC
CGTGGGCGTGGGACTCTCCGAGCCAGGCNCGGGATATAATCTCTGATATGCCGTTTGTGTAAGACCATTGGAAAAGTGC
AGTATTAGAGTCCGAGTGACCTGATTTTCCAGGTGCCGTCTGTACAGCTTCCGTTGGCTAGGAAAGGGAATTCCTGA
CCCCTTGTGCTTCCGAGGTGAGGCGATGCCTCACCTGCTTCACTCAGCTCAGCTGAGGAGGAAAGCAATTTCAAGCTTGT
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GAAACATGCTGTATATAAGAAACAATATGCCTACATCTCCGTCTCTCTTTCTGTGTGTGTGTGTATGTATGTGTGAAG
TCATATGTTTTATGCTTTCTATATATCAGATTATGTTTTAGCATTTTTCAGAGGCACTGTGCTCTGCTAAAATCCTGTGT
TTCCAGATGAAATGGCAAACATTATTTCCAGCAATGTGATAAAACAACCAAGAAAGTGTTCAGGTGACCCGATTTCATA
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ACCAAATCCAGCCTACTGTTTTATTGTAAAAAATTACATTTGAACACAGTCTCACTACCAATTTATTAATCTACTGTTTAT
CACTGCTTTTGTCTAGAAAAGCAGAGTTGAGTAGTTGAAAAAAGACTGTGGCCAGGCACAATGTCTCATACCATAAT
CCCAGCACTTTGGGAGGCCAAGGTGGATCACTAACTGGTCACTAACTCTTGGGCTCAAGTGGGTGGATTACTTTAGCCC
AGGTGTTAGTGACCAGCCTGGGCCACATGGTGAAACCTGTCTTCTACAAAAAGTACAAAAATTAGCCGGGAGTGGTGGT
GTGAACCTGTAGTCCAGCTACTGGGGAGGCTGAGGTGGGAGGATGACCTGAGCCTGGGAGGTCAAGTAAGGCTGCAGT
GAAGTGAATCATGCCACTGCATCCAGCCTGAGTGACAAAGTGAGATCCTATCTCCAAAAAAGAAAAAAGAA

Fig. 6.16

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CTGTAATATAAGACCCCAATGTCAAAAATATTTACTATCTGATCCTTTACAAAAGCGTTTGCTAACTCCTGTCCTTGT
GTATGCTTTGATTTTCATATTAATTATATCTTATGTTATTTATGATACATATATGANATACACATATATCTAATATGT
TATATATTTAAGAATTATTCACATATGCCACAACTGTAGATACAAAAGAAGTATCTCTAGGGAAAGCCAAAAACAACA
TGGAAAGAAAGAAGGCTAGCAGAAGCTTCAAAAATATCAAAAACCTCTTACTGTGTGGCAATATAAACTAAAACTGAT
GCTCAAAATCATGAAGATAGGAAAAAGAATCAAGACAACCAAAAAATATGGTTAATAAAAAATGAAGACAGAAGACATCA
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AAGTACTGTCTCTTTTGTATGAGCCATATTTCAATCAACAGTGTTTAATGACTTTTTTCCAAATCATTATTTATTTTGT
TATTTACTGAGAATCAAACAGAACTTACAATAGAAAAAGAGATACTTCTTAATTAGATATTTTGGAAAAAATCATTG
TCGTGTGACAAGACAACCAAGAGTAGTCAGCTAAACCTATAGAAATGAAGTATTCTAAAAATGTGTCAGACAGCTAAT
TAACACAATATTTTATTTTTCTAAAGATCTTGCAGTGTGTGTCATCTTTATCACCTTTTAAAGTTTGCATTTTATTGT
CACTTTTAAAAATTTTAAATAAATGTCCATTTTGTATCTATCATTCGTGTCTTTATGTAATAGTATCTAATATTTCTAT
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GCATTTTTCTGCATTTTTCCAGAAGTGAAATGCTTTGCAACATCCTCAGAAAGAAAGAGAAATACATGAGTAATATGAAA
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TAACTGTAGAACTTTTTATTTATTTATTTTAAAGGAATGGTTACTATTCTTAGGAAAAGTGGCAGTAAATATAGTTAA
TAACGGTAATATAATTTTATAAACTCATTCAAAGTCTTGATTTTAAAGGCGATAGTAAAAAATATATATCTATTT
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CATCATCTTCAGTATTCATGTCAAAGATTTATTTCCATTTTGCAAAATTTGCTTAGAACTCACCTATATTTACCTTTT
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TCTTTAACTGAATTAGAATTATTGCACATTACATTGCATTCATCAAGGCCTACCCTTCAGGCAGTCTGATGTAACAG
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ATCTTTATTTATCCATTCATCACTTTGATGAATATTTGGATGGTTTTTCACTTTTGGCTACTATGAACAATGCTGCCAT
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CAGTAAGTATGGCATGAGGATTCCAAATTTCTTACAGCCTTGTAAGGAAAGAAAATTTTACAATTATAATTATTTGGCAA
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TTGTGGAAGAAAATTAGAACTTTCTAAAGGACATTATTTTAAAGTTTATTACTTATTATTTATTAATTTTTATTAATA
CATAATATTGCTATGATCAGGTACATGTGATAGTTTGATACATGCATACAGTATATAATGATCAAATCAGCATATTTA
GAAATCCATTACCTCAAGCATTTATTTCTTTGTGTTTGGAAACATTTCAAGAACTTCTTTTCAGCTATTTTGAAATAG
CAATATATTTTTTGTAACTATAGACACTCTATTGTGCTATTAAACACTAGAACTTATTTCTTCCACATAACTGTATGT
TTGTACCCATTAAACCAATCTGTCTCATCCCCCAGCCCTTCCAGCCTCTGGTATCTATCATTTCTATTTCCCTACCTC
CATGAGGTCACTTTTTAGCTCCACATATGAGTGGGAACATGTGATCTGTTTTTCTGTGCTGGTTTTATTTCACTA
AACATAATGACTTGCAATTCATCCCTGTTGGCCGATATGATGAGATTCATTTTTTAAAGGCTGAATAGTATTTTGTG
TGTATATATACCATATTTTCTTTATTCATTCATCTGTTGATGGACACTTAGGTTGATTTTCATACCTTGGCTGTTGTGAA
TAGAGCAGCAATAAATATGGGGGTACAGTTGTCCCTTGATTTTATGATTTCTTTTCTTTTGGACAGAGAGACAGTAGT
GGGATTGCTGGGTGAGTGGTAGTTCTATTTTTTATGTTTAAAGACACTTTCATACCTGTTTCCATAGTCGTTGTACTA
ATCTACATTTCCCAACAATGCATAAAGAGTTCCCTTTCTCCACATAATCACCAGCATGTGTTATTTTGTGACTTGTGATA
ATAACCATTCTAACTGGGGTGAGATGGTATCTCATTGTGGTTTTGATCTGTATTTTCTGTGATCCGTGATGTTGAGC
AGTTTTTCTTAAACCTGTTAGCCATTTGTCTTTTGAAGATGTCTATTCATGTCCTTTGCTCACTTTTTTAGTGAGATTA
TTTGATTCTTTGCTGTGCAATTTGTTTGTGATTTCTATGATATTCTGGATATTAGTCCCTTGTGGATGAATAGTTAGCA
ATTTTTTCTCATGTTTCATGTTATCTCTTCACTCTGTTGATTGTTTCTTTCTGCGCAGAAGCTTTTTAGTTTAAATG

Fig. 6.17

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TCGTTCCATTGTCTATTTTTGTTTTGGTTGCCCTGCTTTTGAGATCTTAGCCATAAAATCTTGCCTAGATCAATAT
CTTGAAGCATTTCCTCTATGTTTTCTTTTAGTAGTTTTATAGTTTTAGGTCTTGTATTTAAGTCTTTAATCCATTTTGA
ATTGATTTTTATACATTGTGAGAGATAGAGGTCTAGTTTCATTCTCTGCATGTGAATATCCAGTTTTCTTAGCACAAT
TTATTGAAAAGAAATGTCCTTTCTCAGTGTATGTTCTGGCAACTTTGTCAAAAATTGGCTGGCTGTAAATATGTGAAT
TTATTTCTGGATTCTCTGTTCTGTTCCACTGGTCTGTGTATCTGTTTTTATACCAATAGCATAGTGTGGTGTCTAT
AGCTTTGTAGTATATTCTGAAATGTGTAGTGTGATTGCCTTCAGCTTTGTTCTTTTGTCTGAGTATTGCTGCTATTTG
GGCTCTCTCTATGGTTCTATGTGAATCTAGGATTGTTTTTCTATTGATTCAAAGAATGTCATTAGTATCTTGATAGGA
ATTGCAATAAATCTATAGCTTACCTTGGGTAGTATAGTCATTTTTTAAACAATATTAAATTGTCCAATTCATGAGCATAAT
ATGCTCTTTCTATTTTTGGTATCCTCTTCAATTTTGTTCATCAGGGTGTAGTGTTTTTGTTTTGTTTTGTTTTGGCTTT
CATAGAAATCTTTACCTCCTTGGCTAAATTTATCCCTGAGACTTTTTTGAAGTTATAAATGAGGTGCTTTCTTGGTT
TCTTTTTTCAGATAGTTGGTTATGGTGTATAAAACACAACCGATTTTTTATATTGATTTGTGTCTTGTAACTTACT
GAATTTGTAAATCATGTCTATCTGAAGTAGAGACAATTTGGCTTCTTCTTTTTTTTTTTTTTGAATTGGAATCTCTCT
CTGTCTATCCAGGCTGGAGTGCTCTGGTGTGATCTTGACTCAGTCATCCTCTATTTCACAGGCTCAAGTGATCCTCCCA
CCTCAGCCTCCTGAGTAGTGTGGGACTACAGGTGTGTGCCACACCTGGCTAATTTTGTATTTTTCTCATAGAGACAGG
GTTTCATCATGTTGTCTAGTCTGGATTGAACTCCTGGGTTCAAGCAATCTGCCACCTCAGCCTCCCGAAGTGCTGGG
ATTACAGGCTTGAGCCACCACAACCTGGCTGGCATCTGTTTTCCAGTTTGGATGCTTTTCAATTTCTTTCTTTCCGGA
AAGAGAAAGTCTGGCTAGGACTTCCAGTATAATGCCGAATAAGAGTGCTTAGAGTAGGTGTCTTGTCTTATTCTAGTT
CTTAGAGGAAAGGCTTTCAGTTATTCCCCATTAGTATGATGTTAGCTGTGGGCTCGTCATATATGGCCTTTATTATGT
CAAGGTATGTTCTGCTATACCTAATTTGTTGAGAGTGTTTCATCATGAAGGGCAGGGTGAAAGGGATTCTTTCTGAAG
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TACTATAAAGAATTTAATCTGGCAAAATGTGATAAATCAAAAGGGTAAATTTGTATGCTAAATAACACAGAAATAG
CAAACATAGGGAAGAGCTGCTACTTCTAGGACTGAGGGAGGATACCCAAGAAAAGAACAAACATGGAGGGTCTTTTAC
ACCTNAAGGCTGAGATTAGACCTTGTGGAGAGGGTGGTGTGTGGCCCGCAGGATAGAAAAGTTCTTTGAGGTGCCA
CAGGCCAGGCTTGGTGAAGTAGGGTACTGGCTGTTGGGTGCCAGTGGATCAGCACTGTGGTCAAAAAAGTGCTTCTAGG
GTGCTGGAAAAACCACTGGAAGGTGGTCAACATTGGGTCTCTGTCATCTGCTGGCAAGGAAATGCTGCTCGGGTG
ACAATAAACTCAGTAGGAAGCCCTCACTAGGTGCTGGTGGAACCTCACTGTAGGGTGACTCTCCCATACACCCTGGTG
GCGGCCACAGGTAAACAAGAACAGGAAGAATCAAGAAGGAATGCTCCTTTATTGCTATACCTTGTTTTATTTTATTTT
ATTTATTTTATTTTTTTGAGACAGAGTTTTGCTTTTTTCCCTGGCTGGAGTGCAATGATGCGATCTTGGCTCACTGCAA
CCTCCACCTCCAGGTTCAAGTGATTCTCCTGCTCAGCCTCCCGAGTAGCTGGGATTACAGGTGCGTGCTAATTTGT
ATTTTCAGTAGAGATGGGGTTTTTACCATGTTGTCCAGGCTGGTCTCAAACCTCCTGGCCTCAGGTGATCCTCCTGCTCT
GCCTCCCAAAGGCTGTGATTACAGGCATGAGACACCGCATCTGACCCCTTACTATACCTTGTAGTGTCTCCCTTACA
CTCTACCAGCAACAGATGACATTGCACTGGCTGACCGAGGAGCCAGATTAGTATCGTGGAACAGGGCAAAGAAGGGTGG
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AATGAGGAGACACAGTTCACACAGTTATCGTAGTCTCATCTGGCTGTCTTAAATACTCCTCAAATCAAAGTCCCACT
GAATATTCTCTTACCTAAAGGCTAAATTTGTAGAGTTTATATTCAACAACCTTTCATAAAATAATGAAGAGAGAAAAGGA
AAATGGTTAATATACAAATACACACATATACATCAGTCAAGAGAGGAAATACACAAAACCTGTGAGAAATCTCAGTT
TTGTAATTGATTGAGAGGCCATAGTTGGTATCTATGGCTTCTTCTTCTACTGCTATTCTGTATTTCCATTGCCCTTA
AATGGTCAAGGTTCTTTATCTGGGGAGTGATCCAACTTTTCAATTTCTCAACAGCCTGAATCTCAATAATCTGCCCCC
TCCTTTTGACTCCTGCGGTTTTCCCATTAACCTTTATTTACACCTTGAGTCCAGGTTCCAGGGAGTTAGCTAATATTGG
AGATGACGATCTGGTCGTAGGACAGGTGTCTGGTGAATGCTGTTTTTCCCTTTGGTCTTGGTCTTGGACCTCATAC
ATTGCTGTTGAGCCATTGCTTGTCTGTCTACATGGCATATTACCTCAGGAGGCTCTTCCACTATCTTCATGTGAT
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AAGAGGGATCCAGAATCCCTGCACGGAATCCTCTGGCTGGATGCCAGATGCAGCCACTCTCTTCCCATCTCTGTGCTC
CTTTTCTGGGTGGTGGTAGGGTGGGTGGGAAATTTCTTGTGGTCTTGGATCTTGGCAAGACATCAGTACAT
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TGCTTTTGTCTCAATTTACCCGTCCCTGAAGACAATAAACAAGAGATGGCATTCTGCTTATAAAAGGGTAGTATAGT
AAAAAATGAAGAAAATATATCTCACAACCTCATTTCCTATCTTTTAAATAAGTATAGACTTAAATAGCTAAGATTG
AAAATGATGCTTATTGAAAATTTACTAAGTAATTACATTCTAGCAGTTATACATGAGATATTATTATATATACATG
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CATTTGAAATTGGATTGAGAACACGGAACAAGCACAACCTTAAAGTGTGAGACATGCTAGATTTCTTCTCGCTCCCA
TGGA AAAAGTAAACACAGCATAAAACACTCATCAATTTTGAAGTTAAGAATGTGCTTTATGTAGTTTATCTAGAAA
AATCCTCAAGTTATATAAACTTTTAGATTATGAATGAGTTATGGTCAATTTGGAGATGGCAACAGAGCAACTGGCAGGTT
CAAGAAATTATACACAAAGGTGAAAGGCTTTTTGAAACAATCAGAGTTTTCTTTCAAGTCTAACTTCTGAGTAATTTA
TCTGAGAAATTTGTAGATGACATGCAGGAGGACTTTCAAAATCATCCAGTGTCCCTCAATCAATCTGGTTCAAATATG
CAGAATTGGAGGACAGGTCAAGAAAGCAGACGCTGAAACAGGAGGAAATGAAGTCGGCTTATTGTGAACATGACTTTTT
CCCATCTAAAAAAGATGATAGTTTACATTGTTTTGAGAATTGTGGAGTGGGAGAGCAGACCAACCTTTCATACTGCT
GAAATATAATTTTCTGGCCACACATCTTCTCATTAATTGTCTCCAATTCCTTACAAAAGAAAAAACCCTTCTTA
CATTTTTTTCTATTATAAAATAATTTTCATAGTTGTATTTACCAATGTTTAAGCATGAATGTGGTAACTGGTGAACA
GCTTAATCTGTGGTGGCATGAAATGATGAAAAATAAATATATTTCTTTTTTTTTTCTGTTTTTATCTCCATCCA

Fig. 6.18

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CATCAACTGAAGGGTAGCTAGATGGTTTTGTGCGAAGTCTGAGTGTCCCTTGTGCCAGGAGATGCTTTTGTACCTAG
ATCTGTGCTTCTGGAGCTACTTCACTGGATTGATGTTTTAGGGAGGACTAGAAAAGTTGAAGGGGAGAAGTTGAATCATGG
AGGATGTTGGAGTGTAAAGATCCCTGAATGTGGTACCTGTAGATAAATACAGTGACTTTGAGACGCGAGCAAGACTC
CAGTGGAGTCTGTTATTCAGTGAGCAACAGCGTCACCCACCATGGAATAAATCAGGTTGGGGTGGACAATGTATTTCAA
GGTTCTGTGATTAAAGATTGTGGGACACACCAGCGGCCCTTCCATAAATAGATTTTACAAGCCTCAGCCTTCTTATGTT
GGTGGGTGGGTAGATTGGTGGCCAGAGAATGACTGAGATTGGGTTTTCTGTCAACTCGAATGGGAGAGGCTCCTTCAGT
TGGAATTAGATATGTAAAAGGTAAGAAATGTGTTATTTTCATGTACCTGTGGATTGTGAAACAAATTCATGCTCACTAC
ATTTGTTTTCAAAGCATAAATAGGCCCTGCCTTCTGCCTTATCTACTCTACAGTATAGTATGGAACAGTATGATTAAG
GCCAGTATGATTTGAGCCAGAAGATTAAAGTTCATAAAAAGTGAAGAAAAGTCAACAATTTAACACAAGAAATGTTTAC
CGACTAACTATTCTATGCCAGGTCTGGGAGTATGAGGAAATAAATGTGACATATCCCTTGAGAAAGCAGAAATAATCCA
AAGAATTCATACATATACAAATTATTAATTACATCAGTAAAAATCTTTATGATGGGTGTGGCCAGGTCTGGGGCTGGAA
GGCCACCTCAGCATTGCAAAAGAATTAGTCTTCAGTCTGAAGCTCACAGAATTTGCTAACCTATTACTTAGGGGGAA
TATACCCTGAACCTATTACTCTTCTCCTTTAGATTAAATGATTAACCTTCTGATAATGCTGCAGACATTTTTTTCTGA
CTCTCGAAGAAAATCAGACCTGTAGTTCTAACCACTCAACTGACCTTAGTTGAGAATGTTAATGTGCCAGAGAACAA
TGAATGCTCACCTTTCCGAGGAAGACAGTAGAAAATAACCCGCTTGACTATCTTCAGCTGGAAAAAGAAGCTTTTTTT
TTTTTTTTTCTGTGACGGAGTTTCACTCTTGTGTCAGCCTGGAGTGCAATGGCACAATCTCAGCTCACTGCAACCTC
TGCCTCCCGGTTTCAGGCAATTCTCTGCTCAGCCTCCCGAGTGGCTGGGATTACAGGCGTGCACCACCACGCCCCGC
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CACCCACCGCAGCCTCCCAAATGTCTGGGATTACAGGCGTGAGCCACCGTGCCCATTCGAAAAAGAAGCTCTTTAATCC
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CCAACCAAGAGCCTTGGAGGCACCAATGATGATGATTTTTCTCTAGTTCTTCAAAGCCAGGAAGTGGGCCATATTGTT
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CTTCAAAGAAAGTCACTTTCTCTCATTTGCATGTCTCTCATTTCTAAGTGACTTAATGAAAATTCCTTTCCCACTAA
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CATGTTCTGTTTCAATGTTGTGAACATTCAATTTAAACCTACTATATTTAGCAGAGGTTTAATTACCCAAATTGGGAAA
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ATCTCTCTGTGGCATTACAGATATGTGAGATGTTGAGGAAGTGGGCTGTAATGATGAAATTTCTTGGATTTTTCAGC
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TGCAGTCAATGGATGAGTCTGAGGACAGGAGACCACTAGCAGGAGAATGGGCAGTGCCAACCTGGCTTTAAAAATACTCAG
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TCTGAAGGATCATTTTATGAGTATCGTAACAGGAACCTGATGTGATTGATCAGTGAAGCAATGGTGTCTTGAAGTGA
GATCCACAGTGGTGCTTCCCAAATTTATGTGCATATGAATATCTGGGGATCTTGTAAATGCAGATTCTGTGCTGCT
TCTGGGGCTTGGGGAGGAGCCTAAAATCTGCATCTCTAATAAGCTGCCATGTGATGCTGATGCTGCTGGTACCAAGA
TTCTAAAGGACAAACATAACGGAAAGCAGGGGTTGGGGGAAATAAGTTTTCAGGGAGGGAACAGTTATACACAGCAAG
AACATAGTCTGCATGGTGTCTTCTGGTCCCTAACCACTGAAGCAAGGTCAAGTAATTGTTCTACCTTCTCCATGAC
TAATTGGCAGGATATATGTTCCCTGATAGGACATTTCCCACTTTAAGGCTGCTCTTTATGTTTACTTCAAATTCCT

Fig. 6.19

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TTTACCTCTTGTAATATTGGGATTTCCATTTAAAGGTCAGACAAAAGATATGTCTTCTACCCTTCTCCTTAGAATTT
 GCCAGAAAACAGGGACAGAAATATGAATTTTCATATTTGATAAACCTTGAAGACTAACTCCCAAAAGATAATATTTGGAG
 AGGCTCTCAATGCAGTGAAGATCAATATGGGTGCACAAAGGCTCCTGGAGGGAGTGTCTGGGAACACAGGTCTCAGAT
 AAAGCTGGCAGGAACTGTTTTCTTAAGTAGATGGCACTTTGAGGGCAAAACCAACCACCTTCTTCGGAATCACAGG
 AAAGGCAGGTACATGGAGGGTAGGGTTGGAGCATCCCTTTACCGCATTTGACCTCACAGGAAAAGTGGAGCAAGCAGAAC
 TGCAGAAAATGTGCAGAAATGCTGCAATCAGTCTGTGTCTAGTGAGGCAGGGTGGAGGAGAGAGGCTGAATTGCCCTCC
 TGCTCTGTAGCTGCCAATGCCCTGTGGCTGGAGAGAGGTAAAGTCAAAACAACCCGCACATATAGTCTTGTCTCATCATG
 AGTTTGTCCCTGAACCAGGGAGAAATCTTGTCTAGCCTGGAACTGCTCATCCCTTCTCAACAAGACCTGGATCACTTC
 CAACATCAGGAAGAACACACTTTCATTTAAAGATGAGTAACAAAAGGCACTAGCACAGGATCTATACAAAATAANTTAC
 AAGACAAAAGGAGGAAGAGGACAGGGGAACATAAAGAATCCTCACCAATAACAAGTTGCCATTAAGTAGAAAATTATGA
 CAAAATAAATGTCTATGAACAATGAATTTAATGAAACAATGAAGAGGCTGGAAGCAGAGCAATTGAGAGATCAGGGGAA
 ATACTAGCACCAACACAGGAGGAAATGAAACAAGAACTTCTGAAGGTCAGGAAGGAAGCAGAAGAGAAAAATAAGCTA
 CCATGGAAATGCAAGCATCTGTAATAAACACTAAGAAGAAACACTGCTGACATCACAGTGAGGAATAGGAGGGCAACA
 TTAAGACACATAAAACAAAAGATATAAGCAAAATAAAGAGGCTGGAAGCAGAGCAATTGAGAGATCAGGGGAA
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 CTCTTTAGAAAATAAATAAGACTTGAATCTACAAATGGAAAGGGCATACTGTAGCTCAGAAAAAATTGATACAGAAATGA
 TCAACATTGAGAGGTATCCAAGTAAACATACTGGCCCTCAAGGCCAATGAAAAATTTTGAATCCAGGCAAAAATAAAA
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 AATTGGCATGGAACATGGTGTCAAAGCTACAGAAAATAGGAATAGGCAGAAATCCTTTAGCAGTAGAAAACCTTAAAC
 GCTTATCTTTTAACTATGAGAAATCATATGAAAAAAGCAAGGATAGTTTTTAATAGCTTTTACAACAACTATATACT
 ATGAAGACAGATTACACTTTCTTTCAAGTATTAATGGAGCAGTTACAAAAATTAACATATATGAAGCCACAAAGAAA
 ACTGCAATGCAGTCGATCACATAGAAATAGTACAGACATTTATGTCATGCCAACGCAAACTTCAAATGTTAAAGAGAGA
 AAAAAAATAGGAAATTTACCTCCTGGAAATTAAGAACACTCTCTATATAATTTTTCAGTCTCATAGAGAAAGTCATCGCT
 AAAAATGCAGAAATATTTGGAAAGCAACACGAATTAACAACTATATTAGAGACTATGAGATCTTGTACAGCAGTACTT
 AGAGGAAAGTTCATACTTTAATACTTTGTAACAAATAAGAAATTAATGAATTAAGCATCCAATATCAGAATGTATTA
 GTAAAATAAAGACAAGGCAGAAATAGAAATTAGTAAAGATAAAAAACAGAAATTAATTAATTTGGAATAATTAATAATGC
 CAAAATAAAGTCTATGAGTTGTGTTTGAATAATTTTAAAGTAAAAATGTGTGCTATGCTCTTTTTTTGAAATGATACT
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 TCCTTTTTATGTATGAATAATATCTGTTGTACATAACATACCACTCTGTTTATACAATTGATTTTGTATGTGCTC
 TTGGATGGTTCTACCTTTTAGCTATTGTGAATAATGGTGCAACACCCATCTTTTTTTTAAAGGGAAAAAGCAGAAATAG
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 AAGCAATAAATGTAAAAACCTGGATAAAATATGTAATTTCTAGAAACGATAAAGTTCTAAGGCTGGCCCCAGAAAGAGT
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 TATTTTACAGGTTGAGCTCTTCATAACCTTAATGACTCAATCACTCTAAAGCTTTTAAATTTCTCTAGAAGAAAAGAC
 TCTAGTTACTTTTATATAAGTGGAGGTTACACTGTTGTGTCAGTATGCCACAGAACAAACAAAATAATAAAAAAGAAAGTCT
 AATGTCTCTTGTGACTATCAATACAAACATCTTCAATAGAATACTAGCAAAACATATTCCAGGAGGACAGTAGAATGTCC
 TTCCTTTTCAGGAAAGGAAGGATAGTTTAAATATTAGGAAATCTATTGATATTGTTTAAACATCCTAGTATATGTAAAAAC
 AGTAAACCATATGCCCCGCACTCATGGTTGCTGAAAATGTTTGTCTATAAAATTAATAATCCATTATTGATTATGAAAAAC
 CACCTCATCAATAGAACTAAATTTATCACATTAGTAAATATATTTGTCTTACCTCAAAGCCAGGTCATGTTTAACT
 AGCAAACCATACACTCACATTAAATTTGGGACAAAGATGCCATTACCACAACATTATTGAACATTGGTCTTGAGGTGCTA
 ACAAACTTTTAGATAAGATAAAGAGAGTGGTATGTACAAGTTGGAAAGGAGGAGTAAGATAATCATATTTGCAATA
 ATGTAACCTCTGTGCCTTGAACCCCAAGAAAAATTAAGTGAAGATCACTGCCAACAATAGTAAAAGAAAAAATAA
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 CTTTCTATGTATGTATGTAAATTTTACATAAAAAATACATGCACGTATATACTTGCAATATACATAGAACAGCTTTG
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 GAATATTAACAAAGTAGATAATACATAGGATGGATATGGAATGATTTGCTTGGATCACTCCAAGCAAGGCATGGA
 ATTCACATGGTTGTGTTTGGAGCTCCAGGGTCTAAACACTGTAAGATTTTCGGAGATTATACACAGATCAGACTCACA
 AACATTTGATTTTCTCATTTTCAAGATTTTCTCCCTCTGGCCTTTCATTTATTCATTTAGTCTTCAGTAGAGAGAC
 CAGGGGATGGCTTGATTTAGGGGCTTAACCACTCCATCTTCATCAGCTCCATTCAGCTGAAGCTGTGTCTTCTTC

Fig. 6.20

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TGAAAAGATCCCAGGAGCTTCCCAATACATATGCAATCTCTGTAAAGCAGTGTGTTTGCAAACACAGCTGCAAGAAAA
GCCTCCTACAGCAAGTATATAAAAAGGTGCTCAATATCACTAATCAGGCAGATGCAAAACCAACAGTATTACCTC
ACACCTGTGAGAAATGACTATTATAAAAAACACAAGAGATAACAAGTGTAGCCAGAATATGCATAAAAGGGAACCTTGT
ATACTGTTGGTGGGAATACAGAGGCCATTGTGGAATAATATGGAGGTTCTACAAAAAATTAATAAATAAATTTCTTAT
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TCAAAGCATTATTACAATAGCCAAGATATGGAACAACCTTTGGCTGTCAATAGATGAATGGATAAAGAAATTTGTGTG
TGTGTGTGTGTGTGTGTGTGTGTGTACACACATACACAATGGAATATTATTTCAGTCTTAAAAAGGACATCTCGCCA
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GTCTCACTTATATGCGAAATTAATAAAAAAAGAAACAAAATTCAAAGATGTAGAAACAGAGTAAAAAGGTGGTTACCA
GGGGTAGGAAAAGTGGGGGAGTGGGGAGATGGAGGTCAAAGGGTATAAAGTTTCATTTATGTAGAATAAATAAGCCTAG
AGATCTAATGTATAGCATGAAGACTGTAGTTAATAATATTTTATTTTCATAAGGGAATTTGCTAACAGAGTGGATTTTAG
GTAATTCCTACTACACACACACACGACACAGAAAAGGTAAGTGTGTAACATGATAGATATGCTAATTTGCTTGATGT
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CACTCATGGGCTCTGCCCTCGTGATCTAATCACCTCTTAAAGGGCCACCTCCAATACCATTACATTAGCAATTAATA
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GTAGATTCTGCTTCAGTCCCTTCTATATAGAACTCTGGAGTTTCCCTACCTCATACTATTGCCTGTTTCCATGG
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CAGTTTCAGATGGCAACTCACACATAGATGTGTTTATGTTGCCCTTTATCAGTATTATCATTGGCAAAGCCTTTGGTAA
GCAACAGAAGTGGCTAAACAGAATAATTCCAGGCTTGAAGTTCCTTTTTCATCTGGTTCCCTCTGGGAAAAGGCTATGA
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TGCCATCAGTTCTTTGAAATGAAACTCTAAGCTGGGAAGAACTCTCTGCTTAGGGGAAGTAGGACTAAAACCTTGACTTT
TGATTGGGAGAACATTCAAAGACTCTAAGCTCATGAGTGCTCAACTTGTAATTTTTTGCCCTTGAAAAACTATAGGC
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TTAAAGAGAGCCGATTGGTCTGTTTTACAGAGAGCTGATTGGTCTGTTTTGACAGGGTGTGATTGGTGGCTTTACAAT
CCCCGAGCTAGACACAAAAGTTCTCTACCTCCCCACCAGATTAGCTAGATAACAGCATCCATTGGTGTATTACAAACC
CTGAGCTAGACACAGAGTGCTGATTGGTATGTTTACAAACCTTGAGCTAGATACAGAGTGCTGATTGGTGTATTACAA

Fig. 6.21

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TCCCTTAGCTAGACATAAAGATTCTCCAAGTACCCACCAGACTCAGGAGCCCAGCTGGCTTCACCCAGTGGATCCCGCA
CGGGGGCCGCGAGGTGGAGCTGCCTGCCAGTCTCGCTGTGTGCCCGCACACCTCAGCCTTTGGGCGGTGCGATGGGACT
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CCACGCCACCCAGAACTCATGCCAGCCAGAACTCGGGCCACCCAGAACAGCGCCGTGCCAGCCCTGGTTCCCG
CCAGCGCTCTCCCTCCACACCTCCCTGCAGCTGAGGGAGCTGGCTCCGACCTTGGCCAGCCAGAAAGGGGCTCCCA
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GCTCTTCAACATCCCATACCTATGCATTTAGTTGCTGTTTCAAAATAGTCACCTGCATATGTTTCATCTTGCTTTTACC
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TGCTTCTGGGTGTTTCAATTTTAGATACCTTAATAATAGTCTCAAGAGTAGCTCAGTTATTAAGTGATTTTGGGGTTGCA
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ACTCACATGCACACATTTCCAAATCAGATATGGTGAATACTAGTGGTGAAGGTTAGGAGCCAGAGGACAGCATCTCAT
CCACTGGGACCTTGCTTCTTACGAGGCTTGTCTGCATTTTCATGGCCTTAAATTTCAACCAATGTAGTTATCTAGTGGTG
CTGATAAATCACTTATAAATTAGTTTTTTATTGTCTAAGTATCAATTTTAGCTGCTGTTTTTTTAACTTTCTTTAGTTT

Fig. 6.22

[illegible]

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CATGCAATTCTTCAAGCATATAGGACTTTGGGTCCTTTTGTGTCTCAGACTTTAAGATGATGTCTCCTGGGGAGTCA
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GACTCTGGGAGGAAGCCTTTTGATTTTGGGGGAATCTTAGGACTATGCACATAACCAGACTTTCCTGATGGGAATCTCC
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TAAAGAGAGTGAATGAGGAGGAAAATCAAATATTGTGGCAGGGAGGGGCTGACAAACACAGGCATGGCCAACAGAAGTC
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TCTCAGCAGCCACTCCAGATGGGCCACCACTGTCTCATCAATTACATTACCTTAGTGTATTGTGAAAATTAATGA
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GTGGCATAGCTCAGGGTAAAGTGGCTCTCCTCACCTCTCAATACCCTACTGTCTTTTGCCTAAATCCAGTTAAGGATG
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TGCAAAAAGCATATATGCATATATTTGCTTGAACCTTGATTTCCACTGACTTGGAGTAGTTTCATTCTCTAAGAATCTCA
TGTCATATATTTTTATATCTTTCTCATTTGTGAAGTCATTCAAGAGATCCTGCCTTGTATGTGTTTTCCAGATAATTTA
CACTTTTATTTTACATAGATGTTGATTAGCTGTGTTTCAATTGAATATTCTCAGTTTTGGGTATCAGTTTTCAGCAAAA
CAACTAAATGTGACACCTTCTACTGAGCATATTGGGTCTATACGTGTGCATTGACTTACGACTTATATTTTCACTTA
AAAAATATTTTGGATACAATATTAAATCTTTTAGCATTAATAGAGTGCTTGAATATGAACCTAGTGCTTTTACTTTT

AATAATTTTAAAAATTTTGATATTTAAAAAATTTGATATTTTAAAAATATTTTCTGTGCTACTAAATGCACTACAATATAA
 TGTGACAATTATGAATATGGATTTTAGATTAAGACAAACCTGGGCTGGAATAGTAGCTCTGTTCCCTTACTAGTTGTGTGA
 TCCTTGGAAAAACAACCTCCAACCTTCTAAGCATTAGTTTCCTTATCTGTAACACAGGGTCCATAATTTCTACCTTACAA
 TGCTGTTTTAAGAATAAACGAAGTGGGAAATGAGTTAGTATCATATTCATATATGGCAGCCATTATTATTATTATTATT
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 TAGGAGAAATTTATCTTATAAAATAAATTAAGTAACCTACTTTTATCTTTAGAGTTTACCAAGATAGGTATATTATTAGT
 AACATGGGAGTCAACAGCNTATAATAAATCTGTATTTCTTAATTTAACAGCACTTTTATGAGTCTCTAACACAAGCTT
 AGCAGTGTTTAAAAACCATGGCAGGGCTGGGCATGGTGGCTTATGCCTGTAATCCCTCACTTTGGGAGGCCAAGGTGG
 GAGGATCACTTGAGGTGAGGAGTTCGAGACCAGTGTGGTCAACATAGCAAAGCATCGTCTCTACTGAAAATACAAAAAT
 TATCTCGGCATGGTGGCAAGCAGCTATAATCCCAGCTACTTGGGAGGTTGAGGCAGGAGAATTGCTTGGATCAGGGAGG
 TGGAGGTTGCAGTAAAGAACTCGCCACTGCACCTCCACTCCAGCCTGGGCGACAGTGCAGAGCTGTCTCAAAGAAAAACAAA
 CAAAAACAAAAACAAAAACAACTACCCAGCGCAGGAGGAATTTCAAGCATGTGAAAGCTGTTACCAAGGATAAATTGTG
 CCTCCATCACAGGTGTCTGCCCTCCCCATCTCTGCTGAGTGTGGAACCCACAGTATCACTTGTCTGGGTTTTT
 ACAGTAGCTGCCTTACCCTTAACCTCCCATTATTGTCTCTCCAAATCCATCCTTTATACCTTTTTCCGAAATATCTTTT
 CTAACAAAAAATCATGCCATCATCATTCACACATTCATAGACAGCTGTTGTCTGTGGAAAAACCTTCAGCCTCATTAAAG
 ATGTAAGGCCCTCCGTACTCTTGGCCCTCCAGCTGTATCACCTCCTGTTTTCCTTCTGCAGCCTATACTCCGGCCATA
 GGACTAATTGCAAATCTCCATTAGTACCGTGTCTTCCCAGCTGTATTATTTAGCTAGACATGTTTTTTTAGCCATTTCT
 TGCCTCTCAAAATGTACTCATCTCTTAAAGATAAAGAGAACCACTTATCTATAGTCTCCCTAGATGTAGAGGAT
 GAATTCGAGTGTAAAGCAAGAGGTGGGCGTGTAGTGTCTTAAGGTAGGAGACTAGGTATAGAAAGAAACCTTGAG
 ATGGAACCAAGGACAGAGAACTTTTGCAGAAATGCTGTCTCTGTGGGCTGCCCTGCTTCTCAGCTGGAAGTGGT
 TCTTCTTTCTGTGTTCTCTTCTCTGTATGGGCTGCTGAGAATTATTGCATGTAGGAAGCCAGAGAATGTCTCACTGTT
 CTCCAGCAGCTGCTGCTTAGGGCTCTCTTACTCCACTCTTTTTGATTCCCTGGTCTCCTGCAGAGCCATTTATTGTC
 TGGACCTTCCCTATACGTTGTCTTCCCCTTAGCTCAAGGCCTGGCCTCTCCTTATCTCTCCTCAGAGTTGACTTCTGA
 TGGGATCTGTTGTGTCTGGAACCTAGCTGTGATGTTTCTCTTCTCCTGCTTACCCTATGGGTTGGTTTCTCAGCCCTTAT
 ACTGTTCCCCTGGCAGCCGCTGACCCGATTAATCCCTTTCATCGTCTCCCATACCCAAAGGTCTGTCTTGGACC
 AGGAGGCTCACTGGGCGCAAGTATTATCAACAAATGTTAATAAATAACCTAATTTAAAGAAATATCTCCAAGAG
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 GGTCAGGTACAACCTGGGAGAGAAAAATGCAGAAGCTGTGGGCATGCAAGGCCAGAAGTGTAGCCAAGAAGCAGNAGGTG
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 AGGTGCGAGAAATGATCTAATAATAGTTGAATGGAGAGAAAAATCAATGTATGGTCAATCTTCAATTATCAGAGTTATGT
 GTTTCGCAATCCACCTACTTGTCTAAATTTATCTGTAATCCCAAAGCAATCCTTGGCGCGCTTCTGCAGTCATTTGTG
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 GGCTGTAATAAGAAGAAATCTGGCCAGGTGCCCTGGTGCATGAGACCAGCCTGGCCAACATGGTGCAACCCCATCTCTA
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 GCTTGAACCCGAGAGGCGGAGGTTGCAGTGAACCGAGATCATGCCACTGCACCTCCAGCCTGGATGACAGAGCAAGACTG
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 ACTCTAAATAAAATCCTCTTTTGTCTGGTTTATTTTTAGCAGATGTTTACTGTTGTTTACTGGCAAATGTGTTGAGTAA
 GTTAGGTAAGTTGACCTAGACACAGTTTCTGCTCCCAAGGAATCTCAATGTATGAGGAAGACAGGAGATGTGTGTA
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 TGACATTATCTTAGAGCTTCGGGGATTATAGATGGAAGGGATCCTACAGGCCAGTATGTGACAAGGACCTGAGCATG
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 ATAAATTGGTATGAATTTTCTAAAGACATTTTCCACTTTTGGCACCAGATACCAATTTGTCTTTCAATAATATATT
 CTAAGAAAGCAATAAGAAAAAGGAAGTAATTAGAAATATGTATAAAGATATTTATAGCATTTTGTGTTCAATAATATGAA
 AACTAGGAATCAAAGTGTCTAAGAATACAGTTTAAAAATACACTTAGGCCGGGTGCGGTGGCTCAAGCCTGTAATCC
 CAGCATTTTGAAGGCTGAGGCAGGTGATCGCTGAGGTGAGGTCGAGACAGCCTGGCCCAACATGGCAGGCTGAGGCA
 TGTCTCTACTAAAAATACAAAAATCAGCTAGGCATAGTGGCGGGTGCCTGTAGTCCAGCTACTCAGGAGGCTGAGGCA
 GGAGAATAGCTTCAACATGGGAGGCAGAGGTTGAGCCGAGATTGTGCCACTGCACCTCCAGCCTGGGCGACAGGGTGA

Fig. 6.26

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CTCCATGTAAAAAATCACTTAATATTATGAAAACTGTTTATGACAAGACATGATGTGAAAAATAATCATGGCA
TATATAGCATAATCACAATTTGTTTATGTGTGAACAGAATAACATGTTAACAGAGGTTATCTTTTGCTAATNGGACTAG
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GAAATATTGCAACCATATTTTGTCCANTTGAACATGATTTACTTACGGGAAGTCTATCCTTGTCTTCTAGTTTCTA
CATTTTAAATGTCTCATCATTCAATTTGGCTGAAAGTTAAATGCAATCTCAGTTATTTACGTTAATTCAATTACAA
GCCCTTCTTTCCACAGTATCATTTCACTTAAACCTTCATACTCAGCCTCCTTTGCAACTCTGGCCTCTCTTGCTTT
CCATTCCTTTTGTCTCTGATTAAAAAATACTAAACCTCTGTCTGCTTTTCTTTGACAGCATTAGCCTTTT
TCTCCTCTGCTCAGAATATAATTTTGTATTCACTTTTCAAATATTTACCCTGTTCCCTTTGTTTCTTTATCCTGG
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TACATAGGGTATGATGAGAATGAGTGTATTACGCTGTCATGTATGCATTCATGTTTGTGTGCATGTTGGTGCATACA
TGTGCAAGTTGGTGTGCACTTGTGAGTGAATACATGTTCCAGTCTTCCAGAACAGGAAGTGTGTCTTTCTATATGC
CCTACGCAAGTAGACACTGCAATGATGCTGACTGCCTGTTTGCCTGTCTCTGTATAGCCACCTTTCAGAGCTTGCCATC
TGGATCTCAGACAGTATGCAAGGAGAGGAATGTTTCAATCCACCTGGTACCAACAGGCTGGCATTGTACTTTGAAA
GCTTTGATGAAGAAGATGCAATGTGTGTTGGCTGTGTGGCTTTTTCAGCTGCTTCTGACTGGGCTAGAGACAGGCAGC
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GTCAGAAATCAAGGCCAGGATTTATAGGATGAACATTTTAAAGTGTACATTAGAATGTAAAGTAGGTATTTGTAATTGA
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CAGCCAAGATGGCCGAATAGGAACAGCTCTGGTCTACAGCTTCCATCAAGCTACCAATGACTTTCTTCACAGAATTGGA
AAAACTACTTTAAAGTTCAATGGCACCAAAAAGATCCCGCATTGCCAAGTCAATCCTAAGCCAAAAGAACAAAGCT
GGAGGCATCACACTACCTGACTTCAAACATACTACAAGGCTACAGTAACCAAAACAGCATGGTACTGCTACCAAAACA

Fig. 6. [27]

[illegible]

Fig. 6.28

GGTTTGAGATCAGCCTGGGGAACATAGTGAGACCTGTCTCTACAAAAATAAAAAAATTAGCCAGGCTTAGTGGCAT
GTGCCCTATGATCCCAGCTGCTTGGGAGGTTGAGGTGGGAGGATCACTTGCACCTGGGAGGTTGAGGCTGCAGTGAACCG
TGTTCCACACCACTGCACCTCAGCCTGGACAAACAGAGCAAGATCCTGCCTCAAAAACAAAAACAAAAAAGGA
AAGACAAAAAGAATTGCACAGCGGCCAATACATAAGAGAAGATGCCTAACACCTAACCCCCCAATTAGCATTGTGATTA
TAAAGATAACAATGCTATCTATCTCCCCACATCATTTTGGCAGAAATGAAGTGGTTAAACTCGGTGTGCCCTGTGA
TTTGTATGAAATGGATGTGCTCATACAACCTAGTCATCAACCTTTTGGAGGGCAGGTGGCTGTACTTACTGAAATGT
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ATTTATGTAGTATGTTTGCAAAAGCATATTCATGGGGAAAAATGAGAAATAACTTTAATGTTTATCAATAGAGAACTGGT
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TGAAAAATTATTTAATGGTACAAAACAGGTTATGAAACACAAACATCACATTCTCATACAGTCATATTACTAGAAATAT
GGGAAGTTGTTAATTTAAATCTGAACAGTTAGAATTTACGGCTACCTTTTACTCTCTTTGTCTATTTAACTGTTTCCTCTC
CCACCTATCAACTCTAGTCTCTTAGGGAACCTATTTTAACTTTCTCACTCTGGTGTAAATGGAGATAATACCAAAT
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GACATTCGAGAGCCACTGTGTTATTTCTTAGTGCAACTGTGACTCAGTGTAATCTGCTTTCTTTCCACTCCTCTGATTT
CTGTGTGTAATTTTCATGACAAAAGTTAGACAAAATACCATGGACTGAAGTTAGATATAGAGCAACTCATGTCTCTTTT
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CATGGAGGTGGGACTTACAGGTGTGGTACAGGTTGTATAATCATGATGGTGACCTGCATGGAGAAGGGAAAGCTTGAC
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CATTAGAATAAGTGAGACTTAGCTTAGCTCATTCAACTCCTTGCCAGGCCCCATGCAAGCTTTCAAACCTTTCAAATAT
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ACTATCATATGAATCATGTCTACTACTGTTACCCCTTGTGTGTGATGCTGGGTTAGTGTCTTGCATGACACAGCAA
AATACAGGCGAGGGTTAAAGTGTCAATTGAGAACCGGCAAAAACTGATGATTAATAGTGACCAACCACTTCACTG
TGCTGTTATCTTAGAGGCTTTAAGGCAAAATCTTACTTACAATAATAGTTGAATGCAATATGTGATGTTAGTAGACA
CATTTCAACCATCTGGCAGTTCATTAATTCACCTTCATTTTTTTTTTCTTTTCTCTTTTTTTTTTAACTTTTTTTTTTTT

Fig. 6.29

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ATACTTTAAGTACTAGGGTACATGTGCACAATGTGCAGATTTGTTACATAGGCATACATGTGCCATGTTGGTTTGCTGC
ACCCATCAACTCGTCATTTATATTAGGTATTTCTCCTAATGCTATCCCTCCCTCAGCCCCCTACCCACTGACAGGCCCT
CGTGTGTGATGTTCCCTGCCCTGTGTCCAAGTGTCTCATTGTTTCAGTTCACCTATGAGTGAGAACATGCGGTGTTT
GGTTTTCTGTCTTGTGATAGTTTGTGAGAATGATGGTTTCCAGCTTCATCCATCTCCCTGCAAAGGACATGATCTCA
TCCTTTTTTATGGCTGCATAGTATTCATGGAATTCACCTTCATTTTCAATATTGTGTATATTTATTGACAGTGAGCTTA
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TGAGGCTGGAGTGCAATAGCACAAACCATAGCTCACTGCAGCCTCCAACCTCTGGACTTAAGTGATCCTCCACCTTGGC
CTCCCAAGTAGCTGGGACTACAAGGTGTGTCTACCATGCCAGCTATTTATTTATTTGTTCTCTTTTTCAGAGATG
GGGTCTTGCTGTGTTGCTCAGGGTGTCTCAAACCTCTGGATTCAAGTGATCCTTGGCCTTCTAAAGGGCTGGGATTAC
TAAAGGGGCGAGCCACCATTCTAGCTGACAATTTCTTTTTTTTTTTTTTTTAAATTATACTTTAAGTTTtagggTACA
TGTGCACATTGTGCAGGTTAGTTACATATGTATACATGTGCCATGTGGTGCGCTGCACCCACTAACCGCTCATCTAGC
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TANTATGATTTTATATTCATGGCCTTAGGAAGATTAAATTAACAATAACAACAACAACAACAACAACAACAATAACA
ACAAAAAATTTCCCCCATGTGCCAAGAGCAAATTTGAGGTCCATTTATCCAGATAAAGTGTTTTGTATCTGAACCAA
GAACATGAACCTTTATCTTTATAGTGACCACAGACTCCCATCTCTAGTATCATGATTTTAAATTGAATTAAGCATTTT
TTTTTGCTTTGTTAAGATGAGGCAGGCCTTCTTGCTGCATTTTAAAAAGCAACTATTTTCTTTTCAGTTTACACTATG
AGGCATTGGCTCCAACCTGTGAGCATTGAAACTGTGACGAGTTCCCTACCAGGAACTGGTTCCAAGGTCTAGGGTTTCC
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TTAGTTGATTTGACATACTAACAGGATGGGTCTGAACGTTTTCTATAGTTTACTCATGAGTGACTTCTTTGGCTTACG
TAAATGGCAAGGCCAGACAAATTAGCTTATGGACCTAGCAATCATTCTTGGCCAGATTTTGAGACACTTTTCAATCAA
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Fig. 6.31

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ATTTTCTATGTTAATAATGAAGGAATGACCAATCTGTAGTATATGCAAAAAGTACTGGGTAGAAATATATTAATTTTCT
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[illegible]

Fig. 6. 34

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[illegible]

Fig. 6.35

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GCTCTACACCATGGTTCTCAACTTATTGCGAATACAATGGGAAAAGTGTCTCTACGCCCTTTCTGTTTGAAATTCATTT
TTATCCCTTTCTGTCTGAACAAAACTGTATGGAATCAACACCACCGAGCTCTGTGGGAAAAAGAAAAACCTGCTCCT
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AACCTGTAGGATCTTATTTGGAATTGACTTTCTCTATTGTAAATTTTGTCTCTGCTTATTTTAAAGTTTTCTTTTTT
CACTGTAAAGGAAAGATGATGCTCAGTTTAAACGTGAAAAGTACAAGTTGCTTTGTTACAATAAACTAAATGTATAC
ACATACACACACACACAACACACACACACACACACACAACTTATACCAAATCACACCCTTTAGTTAGTTGTCCA
CTGGAAAGGTGCTCTTTGTTTTCGAGCTTCAAAGAAGCTCGTCTTCTTTTATTGTACTGTCTAGGGACTCCACTGAG
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Fig. 6. 41

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GAAC TTTTGT TTTACATAGCAATCATTTCTGCTGTGTCTCTTTTCCCTACTAGCCTTGGTAGGCTTCTTAGCTGAATTGT
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Fig. 6.45

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[illegible]

Fig. 6.46

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Fig. 6.48

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CGCAAAGATTGGTATCAGTTTACACCAAAACAGAACACACTGCTGGTGGAGCACAGGGAGTTGGAAACAATATACACAAA
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GAGAGGCAACACCAATGTTAAAACCTGATCCACTGTGGTTATTTTCTTCCATTGGGAAACTATAAGGATGCAAAAT
GGGCAGGAGAGTAAAGCAGCAATCACGTCTGCATGGACTGAAGCAATTTAGTTTCTATCAGACATGGTGACAGTGAT
CAATGCATCACAAAATCACAAACACAGATGCCAAGCACAACTGTGTACAGATCCAGATCAAAGATATCTACCATA
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CCTTTTTCATCCTCTTTTGTCTCCAGGAAGTTCCAGAATCCGAATTTAATCATTATCAGTTTGGGTATATTTGCAT
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TGCCTTGATTTCCTCATCTGTAAAATGGAGAGAATACAACCTTTTTTTCAGAAGTTAATTCTTAGTACCAAATGAGCTAA
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CAGCTATACTTGGTAAAAATACCTAAAGCTCTGTTTCATGAAAGTGTCTTAAAAATAAAACTAGTCCCTGGCAATGC
AGCAAATAGCCAAAACGACTTCTTGGTTGATTGGTTTTTTTTTTTTTTTTTCTTTCTGTTACAGTTTTTAA

[illegible]

Fig. 6.54

[illegible]

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CAATCTCATTAAACACAAGGTTTTTGTGGTTTTTCTAAAGGCAAGGTAAGGAAGTCCCTATGACATGGACCATGGTGC
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TTATGTCAGTTGGATTATACAAATAAACATTGTTGTGGGTTATTTAAAAAATTTATTTTCTGAAGCGAGTCTATCAA

Fig. 6.156

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GGTTTTTAAATGTGGTTCTTCATGGACTTCCAAGGAGGTTTAGGATTGGCAGTGAAAAGAAAGAGAAGAATGGGAAGG
ACACTTACATTTCTGCTTCTTGGGATAGAGGTAATCTGTATATTTTGGTTTAAAAACAGATATTTAATCTTTTAGGCA
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Fig. 6.59

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GAAAAAGTGAGCCAAATCTGAAAATTAGTTTATTTTCATATATATTGAATAGTTAGGCCATTTGGCCACACCTGTCTCG
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CTGACAAGGGACTCTGCATTGGCTCTCTTTAATACAGAGCAGAGAAGGAGAAAGGTGAAGAATGGATGTGTGACAGGCA
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GCCCTCTTAAATGCTCCCTTTTGAAGATGCACTATTTATTGTAGCATTTTGCTGAGCCCTTGGGAATTTCTCTCTG
AGAGACTTTTACCAGTATGAAAAATAGCAGCTTTTCTAAGTTTGGAAATATTGTAGTCTTTTTTTTTTCTATTGGTTTT
CCGATTATCCTATTCTCAGAAATAACATTTATTGATCTCAAGGAGTTAAACATTGTCTTGTGTTTCTCTGGTCTGTATT
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GAAAGAAGTAAATGCAAAAAATGGTCAAAGCAGGAGATGATTTTAAAGAGCATCTGGTTCACTCTCCCTTTTACAGC
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ATTGCTCTTTTACTGTTTCAAGGAAGCAACATTTTATAGTTTGAAACTGTTTCTCTTGCATTTGCTTTGCAAGAGGT
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AAGGTGCTTCAAGATCTGCTGTGGCCACTTCTGTGAAAGGGCATGGTCACAGTCACCGTGGCATGGATAGAAAATGG
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CACTTGACTACTTTAAACCAAGTTGACTCTCCTGTAATGCAATAGGGGTTTAAATGATCTCTGTGGCTCTAGAGTTTGT
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TAGTCATGGATGAAAGNAGAACTATTATACCAGGGTAACATCTGGAGCCTGAGATAAAATCCCATTAACAATCTCTTT
AAATATTTCTGTGTTTAAATGGGATGAGAAGACTATCCACTCCACAAANGTAATCCCTTTTCTTCCCTCAGCCTAGTGAA
ACTTATTGTTTCTTTCCCTAGATAAAAAAATAAGGATGCTGTACAGNTTCTTTTGGCTGTTTAAAGACAGA
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CCGTGGGCCCCGGATCCCTGTTTGTCTTCTCAGAGAGCTCACTGGCAGCCTCCCTGATGCTTTGTGCCAGTTTTTAGG
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CAAGGCAGAGGCTTTAGGGCAGGGATCTTCTGTGAGCTGAAATAAAGGGTCTGGTTTGGAGGAGATTGACTCTGCC
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GTCCCTTAGTAGCCGTCTCATTAGCAGATCCACCTTCGAGGTATGGAAGTGCTTGTGTTCAAGGAACCTTTAGTTTACT
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AAAGTTTGAACCTAATAAGGAAAAAACAATAATTATATGCTGAGGTTGCCAAGGTTTATGCTAAAATGAATCTTCTA
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CTTGCTCCTTGGCATCTTGGCGCCTGGGTTTTTGTCTCATTCTGTCTTAGTGTGAGACTTCTACTGAGTCTGAA
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CGGCTACTCACCTTCTTTTATCTACCTATATACTCCCTGCTTTGAAGGCTGAACCTCCAGCATTTTAACTTTTCTGA
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GTGTGTGTGTGTATATATATATATATATATATATATATATATAGTTTGTGTTGTTGTTTTTGGAGACAAAGTC
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GGCATCTGTATTTTATCTGGCAACCCCAACCCCACTGGATGGGTATTGCCAGTGTGGGAACGTGTTAGAATTGAG
GGTAAGTGTGAAATTGGCAGACAGAGAAGGAACTGCAAGAAGAGANCATGGATCTATCAACAGAAATCATTAGCCAT
TCAGACACTTTGTGACAACCTAAAAAGAGAGGAGGAGTGAATTTTGTATGAAGTAAGAATTCAGTTGTTGTACAT
GAACAGCATATGCTAGCCTGCTTTGAAGACTGAAGTTCTTGGCTTTCCAGTTTATAAACCAGTTCTATCTGGGACGCTT

Fig. 6.62

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GCAGCCAAATTGTGTGTGGAGGAATGGGACTCAGGAAGCACGGGCACCCTGAAATAGGTGGATGTGGTCTGTGGAAAA
GGTGAAGCACACACTAGGGTTCTACCCTTAAGAAAATGAACCTTTGCTGAGTTATCAAAGTGAGTACTTGCTATTTCT
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CATTCTTTTCTCGCTCTCCAATAACCTACCCTGTGAGGCTGTCTCCAACCCAGAGCCCTGACCAAGTGACCATGCTG
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GAACCCCTTCTTGACTAGGCCCTTAACTTCTTATCAACTACAGATTCTCAACACCAATGATTTTATCCACTCGTGCC
CCACATTAAAAGACTTACACAAACACTAGAATAATTTCTAACAGCTCAAGGCCACATCCCTAGGACTACCCCTACCCAC
TTAGGATGCCTGCCTGAGGAAGCTCAAGGTTGCCAGGAGAGTTTACTATTTCTTCTAGCCAACCCCTGGAGCTAGGCC
CAACCACCTTTCTTAGAGCATTACTAAAAGGGCTTACAATTGTGAATCCTTGCCCTGTAACCTTTGATATAATATAT
GCACTTCTACTGCTCAAGAGTGTCTTTCTCAAGGACCCAGAGCCATTCTTCTAAAAAGTAACCATCAGGAGAGAGAG
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CTGCTCATACTCCTAGAACAGGCCCTTGGGTGATTTTGTCTATATAGGAGACTGGGACTAGAAAGGTCCAGCTTTCAG
CTGTTTTAGACTATCATTAGGTTGTAAATGGAATCCATTAAAAAATTATATGGAATGCACAAAATAAAGCAGGTGAAG
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GGTGCATTATTATCTTTAATATATTTAAGCTGTAGCCTTGGGGATATTTAGCCTGTTTGCANCTGATTTTAA
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AGCATATTCTACCCTTTGACTTCTNTGTTAGGGATTTTGAAGTCTTCTGCTTCTGACTTTTCGATGAATCATGTCTCTT
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AGGAAAGATGATTGTTGTAGAAACACAATCAGTGAAATAGTCTGGTAGAAAGACTATTCTTAAAAATCTTATACTCCC

Fig. 6.63

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ATAGAAATTACTTCTAAACCATACCTGCCCTTAGACTTGAGCAGCAANAATATTCCCTGCCAGCCNGCATTTCAGAGGATC
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TGCTCACTGACACAGGCTTAACTGGTTAAAGAACTCACAGGTTGTTTCTCACCATTAAATGGCTATTATTTTACC
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AACAAATATAGATGAATATAATAAACATAATGTTGACATAATCAATTAAGAAAGATACACTACTGAATGACTTGATTCCA
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TGTCAATTTCAATTATCAGGTCTACCTGGGACTCTAAGAGAGCAGAGGTGAAATTTTGCCTCTGCTACAGTAGGTTCTAT

Fig. 6.64

[illegible]

Fig. 6.65

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TTGGTTTTCTGTTCTTACATTTGTTCACTTAGCATAATGGCCTCCAGCTTCAACCATGTTGCTGCAAAGGACATGATCT
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ATAGAGATTCTGGTATCTGTATCTTTGTTCTCATTCAATTTCAAATAATTTCTGATTTCTGCCTTAATTTCAATGTTT

Fig. 6. 67

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ACCCAAAAGTCATT CAGGAGCAGGTTGCTTATTTTTCAATGTAATTGTATGGTTTTGGGTGATTTCCCTTAGTACTGATGA
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TTAATTTCTATGTAATCTTCTTTTAAAAAATCTCTCTCTTCTTCAATTTTACCCACTGTCCATTGCTTTGTTAAC
CTACCTGGAATCTCTTCAATTTTCTCTCTGTTTTCTTTTCTATAAAGGCCAATTTATGGTTCTTTATTAAGGACACCAAG
TAGTCTTCTGAGTTTTTCTTTTGGATCTGTCAGTAAAAATTTTTCAGAGGTTTGTGTTGAATTTTGGAGCACTATTCT
CTTTCCCTGTTGTGTTGTGTTGTGTTGTGTTGTGTTGTGTTGTGTTGTGTTGTGTTGTATAGCTCAAAACTAAATACTAGAGAA
TCAAATATATATATATATTAATTAATTTTAAAGTAAAGCCACAAAGATACCCAAGTTGAGTGGGTTTTTTTTTGGTCCAG
CTCTTTTTCTATTACAATGGTTGTGAAACTTAATATCCAGTTTACAGTTTAAATGAATATTGTTGGGTTTAGTTGCCAT
TTCAATTACCATTTTCTGTTAATTTTTATTTAAAGTATCTCTCTCAGTTGGCTTTTGTATCATTACAGCTTCTGCAGCCT
GATTTCTTTGATACTATAAGAAGTTATACACGGTATCTGTAATTTCTCAACCTATCAGGATTGCTCTCTCTGTTCTGTTT
ACCTTTTCTCTCTCCATTTCTTTTTTTTTTTTTTTTTTTTTTGTAGACGGAGTCTCGCTCTGCTGCCAGGCCGAGCTG
CGGACTGCAGTGGCGCAATCTCGGCTCACTGCAAGCTCCGCTTCCCGGGTTACGCCATTTCTCTGCTCAGCCTCCCG
AGTAGCTGGGACTACAGGCGCCGCCACCGCGCCCGGCTAATTTTTTGTATTTTGTAGAGACGGGGTTTACCTTGT
TAACCAGGATGGTCTCGATCTCTGACCTCATGATCCACCCGCTCGGCTCCCAAAGTGTGGGATTACAGGCGTGAG
CCACCGCGCCCGGCCCTTCTCTCTCATTTTATCAACCAAGGCTCTACCTCTGCAGACTAAGCCTTTTGAACATGTG
GCTTTACGGGCTCTCTCCCTACTTTCTGCTCACTTTTCTTTTCTTAAATACGCTGGAATGGTGGGAGAAAGAGCAATT
TGCCTCCAAGTAAATTTCCCAAAACCAAGAACTCTGGGCTCCCTTTTATCCAATAAAGAAAGAGGAGTGTGTTGTTG
GCAGAACATATACTAACTTGAACACACAGAGAAGATTAGCATGACCTTACACAAGGATTACATGTAAGTTCTCTGA
AGCGTTCCATATTTTCTTACGTAATAGTAAAGGTTGATTCAACAAGAAGAGATAACTATCTAAATATATATGACACC
CCAATACGGGAGCATCAGATTCATAAGCAAAATCTTAGAGACTTACAAAGAGACAGACTCCACACAATAATAGTAG
AAGATTTTAAACCTCACTGACAAATTTAGACAGATCTTAGAGACAGAAAATTAACAAAGATATTAGAACCTGAACCTC
AGCTCTGGATCAAGCAGACCTGATACATATCTACAGTATTTCCACTAAAAACAACAGAAATATACGTTCTTTTATTGTC
CATACAGCACTGACTCAAAATTAATCACATAACCGGAAGTAAACACGCTCGCAATGCAAAAACAACTGAAATCATAA
CAGTCTCTCAGATCACAGCACAATCAAAATCAGAATCAAGATTAAGAAATCCAGAACTAGAGGCGGGCGCGGTGGCTC
ACGCTGTAAATCCAGCACTTTGGGAGGCGGAGACGGGCGGATCACGAGGTCAGGAGATCGAGACCTCTGGCTAACA
CGGTGAAACCGGTCTCTACTAAAAATACAAAAATATCTGGGCGTGGTGGCGCGCGCTGTAGTCCAGCTACACGGG
AGGCTGAGGACAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAAGCGGAGATCGCGCACTGCACTCCAGCCT

Fig. 6. 69:

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GGGCGACAGAGGGAACTCCCGTCTCAAAAAAAAAAAAAAAAAAGAAATCCAGATATAACCACACAATTACATGGCAAT
TGAACAACCTGCTCCTGAATGACTCTTGGGTAAATAATGAAATTAAGGTAGAAATCAAGAAGTTCTTTGAAACTAATGA
GAAAAAGAGACATCATACCAGAATCTCTGGGCCGTAGCTAAAGCGGTGTTAAGAGGGGAAATTTAGGCTGGGCACGGTG
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CCAACATGGTTAAACCCCTGTCTCTACTAAAAATACAAAAAGTAGCTGGGTGTGTGGCAGCACCTGTAGTCCCAGCTAC
TTGGGAGCCTGAGGCAGGACAATCTCTTGAACCTGAGAGGCGGAGGTTGGAGTGAGCGAGATTGTGCCACTGCACTCCA
GCCTGGGTGACAGAGTGAGACCCTGTCTAAACAAAACAAAACAAAACAAAACAAAATTTATAGCACTAAAT
TCCCACATCAAAATCTGGAAAGATTTCAAATTAACAATCTAACATCACAACCTAAAGGAACTAGAGAATCAAGAGCAAA
CAAAACCCCTAAGCTAGCAGAAGACAAGAATAACCAAGATCAGTGTGAATTGAAGGGGATAGAGACAAAAAAATCCT
TCAAAATATCAACAAATCCAGGAGCTGGTGTTTTGAAAAATTAATAAAAGAGACTGCTAGCTAGACTAAAAAGAAT
AAAAGAGAGAAGGATCAAATAAACACAATTAGAAATGATAAGGGGATATCACCCTGACTCCACAGAAATTCAAATAA
CCATCAGAGAATACTTTAAACACCTCTATGTATATATATTGGAAAAAAGTAAATTCCTGGACACATA
CACCTTCCAAAGACTGAACCAGGAAGAAATGAATCCCTGAACAGATCAATAACAAGGTCTGAAATTGAGACAGCTAGTA
AGTAACCTACTAACCAAAAAAGCCCTGGACTAGATGGATTAACAGCTGAATTTACCAGAGGTACAAAGAAGAGCTGG
TACTATTTCTGCTGAAACTATTCCAAAAAAGGAGGAGCTCCTCCCTAATCTATTCTATGAGGCCAGCATCATCTCTGA
TACCAAAACCTGGCAGAGATATAACAAAAAAGAAAACTTCAGGCCAATATCCTTGATGATCATTTGATGCAAAAGTCTCT
CAATAAAATGATGACAAAAACAATCCAGCAGCACATCAAAAGCTTATCCACCATGATCAAGTTGGCTTCATCTCTGGG
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TTATCTCAATAGATGCAAAAAGGCTTTGATAAAGTTCAACATCCACTCATGTTAAAACTCTCAATAAAGTAGATAT
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AGGATGCCCTCTCTCACCCTCTTATTCAACATAGTATTGGAAGTTCTGGCCAGGGCAATCAGGTGAGAGATAGAAATA
AAGGGTATTCAATAGGAAGAGAGGAAGTCCAGTTATCTTTGTTTGAGATGATATGATCTATATCTAGAAAAAGAT
AGTTTCAGCCCAAAAGCTTCTTAATCTGATAAGCACTTCAGCAGTCAGGATACAAATCAATTTGCAAAAGTTGCTGG
TATTCCTGTACACCAACAGCAGGCAAGCAGAGGCCAAATCATGAATGAATCCCATTCACAATTACTACAGAAAGAAT
AAAATACCTAGGAATACAGCTAGCAAGGGAAGTGAAGGACCTCTCAAGGAGAACTACAAACCAATGCTCAAAGAAATC
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AGTAAATTTATATATTTCATGCTATTCCATTAAATTAACATTGACATTCTTCACAGAATTAGAAGAACTATTTTAAAT
TCGTATGGAACCAAAAAAGAGCCCAATTTGCCAAGACAAGCCTAAGCAAAAAGAGTAAAGCTGGAGGCATCATGCTACC
TGACTTCAAAATATACTACAAGGCTACAGTAGCCAAAACAGCATGGTATTGGTATAAGAACAGACACAGAAGGCTGGGC
GCATTGGCTCACGCCTGTAACCCAGCATTTTGGGAGGCCTAGGCAGGCAGATCATGAGGTGAGGAGTTTGAGACCACC
CTGACCAACATGAGAAACCCCATGTCTACTAAAAATACAAAAATTAGCCAGGTGTGGTGGCATGCACCTGTAATCCCA
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AACACATTTACAAGAAATAAACCAATGGCCCAATTAAGAAAGTGGGCAATGACATGAACAGACACTTTGCAAAAGAAAA
CATTTCATGCAGCCAACAAGCATCTGAAAAAAGCTCAGCATCACGTCATTAGAGAAATGCAAAATCAAAACCAATGAG
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TTGAGGCAGAAATACCATTTGAGCCAGATCCCATTAAGGCTATATACCCAAAGGAATATAAATCATTCTATTATAAAG
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TACTTATAAGTGGGAGCTGTATGATGAGAACACATGGGCACATGGGGGAACAACACACTGGGGCTGTGGGGGTGGG
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TGCAGCAACACCATGGGCATGTTTACCTGTGTAAACAACTGCACATCCTGCACATGTACCCTTGAACCTTAAAGTT
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TCTCTTGTGTTTATTGCTGACACAAACCAAGAACAAGAAATTTCTTTTATGCCACTGATTTGCAAGGTCGTATAGAAA
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AACACAGAAGGAAGTTAAGGGATTAGAGTGGGAGGGAAATTAGTGAATATCTATTTGTGTCATGATTGCATTTTGGAA
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TCCCAGCAAGGAGTCATCTGTAGGAATATTTGATAAAGCCATCTACATAAAGCCCAACAGCTCACATCATAATTTAA
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CCTAAAAGCAAAGGTAAACCTGATACTTCAAGAACAGACATGACAGGATTGAGAAAATTAACCCCTCAAATTTGG
TAAATTAGACTGCTCATCAAAGACATACGTAATGAGTAAGAGAGCCACTAACAGGACAAAAATTTTGTAAAACATA
TCTGACAAAGGACTTTAATCAATATCACATAAAGCACATCTACATTAATAAGATAAAGACCAAGAAAATAGCTCAATA
AAAATGGGCAAAGCATTTCAAGGGACACTTTACAAAAGTAAATATACAAATGGCCAAATGAACACAGTAAAGAGTGTCTCC
ACATCTTTAGGCTTCAGCTAAATGCATTTACACCACAAAGAAATACCACCACATCCACTAGAAAGGACAAAATTA
AAAGGTTGAAAAACCAAAATCTGGTGAGGAGTTAGAACCCTGAACTCTTACACTGTTGATAGGAAAATTAATGTT
GTAACACTTTTGAATAATGTTTTCAGATAAATAAATGTTTACCTTACCTTTCAGCTTAGCAATTCCTCTCTAT
TGTTTACCCAGAAGCAAATTTTATGTTACACATAAATAAATTAATCATAAATACTTGTAAATATTTCATAAACC

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CACATATTCATAAACCCACATATTAATCAAAAGAGAATCAATAAACAAATTTTGTACAGTTATACAATGGAGTATTAC
TCCGCAACAAAAATGAATGAACCTACTGATACCTGCAACAAAATGAGTGACTCTCACAGACAAAATGCTGAGTCAAGGAA
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ATAGTTCATCAATTTTTTTTTCTGATAAGGGGAGGTTAGGCAGGAGGATTGCTTGAACCTTGGGGGGGCGAGAGGTTTCA
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GTTATATCTAGAATGTTGAATCCTACTTTTCTACTTAAACAGGAATGTTCCCTATGTAACGAAAAAGTCTGTATAATGT
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ATTGCATACAAATTATTAGGGAACATCTGTGTGCAAAACATTTTTATTACATTTTGGATTATTACTTAGAATAGATT
CCGGAAGTGAAGTACCAGGTCAAAGGCTTTTCCCAATTTATTTTTCAAAAAGAAATTCCCACTGTATTCTATACAAGT
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TTCCTTCGAGACAGTGCAGAACATTTGCTTCTTTAGAAAAATTCAGGTCTCTTTCTTTGTGTTACAGCACTTACTATT
TGCTATTGTTGATTATTGGCTTTTGGGCGGTTTCAAAATACACTAGGGGAAAGAGTATTTTTTAAAAATC
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CTGATTGCCTTAGGCTGCAGTGCAGTGGTGCAATCACAGCTCACTGCAGCCTCCATTCCCAAGCTCAGGTGATTCTCC
CACCTCAGCCTCCTGAGTAGCTGGGACCACAGGTGTGCGCCACCATGCACAGCTAACTTTTGTATTTTAGTGGAAAT
GGGATTTCACTATGTTGCCAGGCTGGTCTCAAACTCCTAAGCTCAAGCAATCTGCCACCTCGGCCTCTTAAAGTGCT
TGGATTACAGGTGTGAGCCACCACTCCCTGCCTCTATGATTATTCTATATGATTATTTTATATTAATGTATAAGTT
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TTCTTATTACTTTTGTATCTTTTATTGTTCTAATTTACCTCTCTCAACCCATCCCCCACTGGCACTGTTATATGAA
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CCAGTTAGGGCAAAATGTCATGCTTATTTGAGCTCCTATAGATACATTTAAATCAGACATACGGAATTTGCATTCTG
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Fig. 6. 72

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TTCTCAGATTATCTGCTTTTGAACAACCTTCTTTTAGCTGCAAAACGCTAGTGACGGTGTCTCAATGTGAGACGGGG
AAGATTTTAGTGGGCACAGCAGCTGTTGACTCAAATATAGTTATACATGTTCTTCTGTGTTTCTTGTAGGTGAATCA
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[illegible]

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Fig. 6.76

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Fig. 6.82

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TGTTTATATTTCCAGGTAGAGGCTGCCTAATTAACACTACAGGTTCTAGTGGTTGGGAGCAAGCCCTCTTTTCAAAC
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CACTTCTCTGAGATTTTATAATGGGGTCACTATATGATGCTTCTTGGGATCTGATTTACAATTTATAAATAAGCTGAA
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CGCAGTCTCTTTGGAAAGCTATTTGGCAGTTTCTGAAAACATTAATTTCTAGAGCTGCATATGACCCAGCAGTTTTGT
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GACCAGAGAATTAAGCATTTAAGTTGCAAGAAGTAGGTTTATTCCTTGACTCAAAAAGGCTTAGTATAACCTACTTG
CACCCCTGTAAGCATCTAAATATTGTCTTAAGAGAACAGCAACATAATGATAACCACAGTATTGCTTAATATCTGCTG

Fig. 6.84

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[illegible]

Fig. 6.86.

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CATTAGCTTCATTTTACTGTGATGTAATACTCTATGGAGATTTTCATTTTACTCTTGATAGATCTTTATTTTCATTTTT
CAGTTATGAATGTCTGTTGTGATTTTTGATGAATACATCTACTCATTCTTTTATGTTGACATAGCTAGAGTTAACAT

Fig. 6.82

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GGCTGGTTTGTAGGGTAGACATAATTCAGCTTTAGTAAATGCTGCCAAAGGATTTTACAAAGTGATAGTACTAATCTCT
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Fig. 6.917

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TGAGTATAAGGTTTGAATGTTTCATTTGACCTCACTGATGGCTGTGGAAAAGCCTAGGCTTGGACACAGAACTGAAGG
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Fig. 6.92!

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Fig. 6.95

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Fig. 6.97

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CAGATTTGGAAATTTACATTTTCATATCAAAGGGATTTTCTGTGAGACAAACCAATGAGATTTGATAGATTAGAAAGGAA
GGACTAATAAGAAAGCAACTGAATAAATACTTGAATAATAATGATATGTGTTTTTACACTCTGGTCCAGTTATTTT
TTTTTCTCTTTTAAAAAAATTTTGTAGTGTTTTTGTGTGAGATAGTTAAAGTTCCTGCAATCCACAGAGCTCTATA
TTTGATTAATCTGGATTCCAGCAAGTTTGCATGGCTTTTCAAGGACTACAAAATAGGGAAAAGACTAAATTCATA
TAGAATTGACCCATGAAAATCACGGGAGTTAGTGGTACCAACCCCTGTGCAGCTGAAAATCTGTGTGTAATGTTTGACT
TCTCCCAAAAGTTAACTACTAATAGCCTACTGTTCCACAAAGTCAATTAACACATAATTTTATGTTTTGTATTATAT
ACCGTATTCTTACAATAAAGTAAGCTAGAGAAAAGAAAATGTTATTAAGAAAATCATAAGAAAAGTAAAATAGATATTT
ACCATTCAATTAAGTGGAAATGATCATATAAGGCCCTTCATCCTCTTCATCTTCATGTTGAGTAGGCTGAGGAGGAGA
AAGAAGAGGTGAGGTTGATCTTGCTGTCTCAGGGGTGGCAGAGGCAGAGAAAATCTGCATGTAAGTTGGCCTGTGCAG
TTCAGGCCCATGTTGTTTAAAGATCAACTGTAAATCTTTTAACTTTTCAAATGACGCTCATTCACACAAAGAAAATTTGG
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TTGATTTCTAAGAAGTCAATGCAAGAGGAAGGTGAGAATCAAATTTGGGCAGCTTTGCTCAGCTGAATATTATATGGTG
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TCATAAAGTTGTAGCATAACATATTACTCATATGTTTATGGTGTATGCAGGTGCAAAACAACTCACTGCATGGCCGGTGA
TATAAAGGATAGTACATACAGTTATGTACAGTACATAATCTTGATGACAAGAATTACTGATAATAAGTGTACTGTT
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AAGACAGTGATGTTGATGATCCTTATCCTGTGTAGGCCCTAGGCTAATGTATGTTGGGTTTCTGTCTTAGTTTTTAAACAAAA
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TAAACATGTTTACAATAAATAAGGTTATTATTGAAGAAAGAAAATGTAAAAATAAATTTAGCACAGCTTGGCCTGGCG
CGGTGGCTCACGCTGTATCCAGCACTTTGGGAGGCCGAGGCGGGGCGGATCACGAGGTGAGGAGATCCAGACCATT
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GTACAGTAACATGGCATAACAGGTTTATAGCCTAGGAGAAACAGGCTATACAATATAGTCTAGGTGTGTGTGTTAGGC
TATACCATCTAGGTTTGTGTAGGTATCTCTGTGATGTTGACACAACAACGTAATCACTTATGATGGATATCTTAGAAC
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ATCCTCACAATAACCTTATGAATTTAGTACTATCAATTAAGTACCACTTTTTTCCATGGGAAATAGGATATAGGATATTC
TATCACAAGCATACCTCAATGCTAGAGGAAAGAAAGAAAGAGACGAAGAGGGTGAGAAAGAGAGGAAGGAAGGAAGG
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TGTGTTGATTTAAATAATATCACATTTATTTATTGGGAAAGCTAACTGAGTTGGGAGCAGTGATGTAGGTTCTGTAC
CACCATTAGTTGAAATCATTGCATATCTGAGAAGAAAGACCATGAAATCTAGGAAGTTTAAAGAGTTAGTTTTCGTTA
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TCAACAGTATTTATTGTAGTGAAGAAGTTCAAAAAATTTAAAGTTCAGGAGGACAGTTAAATGAATTATGA
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ATTTTTCAAGTGTTCAGAAATGAACTTTTTATAATGAAAAAAGTTTTAAATATTTTAACTGATTTTGTATTATACTAG
TGATAATCCAGAAGTGATTATGTTTTATACAATAGACTATGGCTTTATATGAAGAAATGAATATAGTCTAGTATTGTTT
TTATTATCTAGGAATATACATGTAAGTGAAGATTTATGAGTAAAGTTAATATAAGCAAGTAACTGGGACTTCTGGAG

Fig. 6. *lec*

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GAAAGCTCACTCTAGGGGAAGCTCTCAAATGTGTTTTCAATCTTGGATTCCAGTCAAAAAAGACATGAGTTACTTGGGA
TTTAATAACCAGATATACATTCTCCTCCTTTAGGACTAGTGAAAAATGGGCACTGAGTGAGCTTTGGGCACAGACTAATA
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AGCATATATAGTAGATGCTTGATAAATATCTGTCAAGTGAATTTCAAATACTTAAATTTTGTGTTAATACATTCATGT
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AGGATGGTCTCGATCTCCTGACCTCGTGATCTGCCCGCTTCAGCCTCCCAAAGTACTGGGATTACAGGCATGAGCCACC
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TTAATAAAGTTTTTGAAAAAATCAACACCTTTTCAAGCAAAAACCTTAAATAACATTAGGTATAGAAAGAAATTTAC
CTACATATAATAGAGGCCATATTTTGAAGAAGTCTACAGCTAAGTAACTCAGTGATGAAAAGCTGAAAGCTTTTTTC
TATAAGGTCTTAATCAAGGCAAGGACATCCATTTTGCCATTTTGTTCACACAGTAATGGAAGTCTAATCAAAGGA
ATTAGGCAAGAAAAGAAGCAAGGCATCCAAATCAGAAAGAAAGTAAACTATTTCTTCTTGGCAGATTACATGAT

Fig. 6. [a]

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CTTATATGTAGAAAAACCTCTTTCTACAAAAATCTGTTGAAACAAGCAAATTCAGTAACTTGCAGAATATGAAATCAA
CATGTTAAACTCACTTTTGTATCTGTACACTAACAATGAACATCTGAAAAGGAAATTAAGAAAAAATTTATCTACAA
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TGACTCTTGTGTAAAGATTAGTTACTTTTTATTGTTTTAAGGCTAGAAACATAGGTTCTATTTTTGTCAATGCTACTA
CATCCCACAAAGTGTCTATATGAGCCATTTCCAAATTTCCATTATGATAAGGGGCTGTGATGCATGAGTATACCCACG
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ACCTAGTACTTAGGGACCTTGTCTATCTATTTTCTGCAAGTACTTTCTGTACTTGTCAAGATCAATAACTGGTTTTA
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TATATAGGATTATATATAAAATATATACTAGTATATATAATATGTACTTGTATATAATATATCTACATATATACACA
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CAAAGTCTTTCATTAATGATACCAGAACATAGGATCTACTGAAATTTCTAAATGGCCAGTTGGAAGAGGAAGGTTCATGT
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TTAATGCTGACCTTCCCTGTTAATCCTAGAAAATTAGAGTTTGAAATAATAATGTCATAGTCACTATTCCTTTAATCT
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GCCTAACGACCTGTCTGTTGAACAAACAAGTTTTCTTTAAGTGATTCTTTGTTTCCATTTATAAGGCACCAACTTT
CAAAGGTGTTCTGGGAAACCTTTCTGTATTTCTTCTAAGCAAAATCAATTCAACAGAGAGTTTACGCTTTGCTGTGA
TCAATGGGGAAGTACCAGTGTAGCTTTCTTTTTTCTTATAGGGCTGCTCATAGTCTCCGATAGACTTTACAGCTGT
TAGTTTTGCTGCAGTAGTGTACTTGTAAATGTTGGCTCATTGAAATGGTGTGTTATTAATTTACACTCCCAACA
GGGTACAAGGGTCTCTCTCTCCATATCTCCCAATATTTATTATCTTTTTTCTCTTCTCATATAGCCATTCAACA

Fig. 6.102

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TCCTTCTATGTATTGTCCATTATAACTTTGTAATTTCTTTTGGGCAGGATCCTGTGATTGGTAAATTTTGTGTTTCCA
TGCAGTTTCTAGGCTAATGCTTTGCATGTGTAGGTACTCAATGTAAAGGTACATGTTTTAAGTGAAAGGACCCAAATTT
CATGACTTGCTGTACAAATGATGATCAGGGTTTAGGAGAGGAGATATAGACCAGAAATCTAGTTGTTGTTTTGAGTTAT
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GTACTACTGGGCAACATTTTATTCTTGGTGGATACAATTTCTTTCTTTTTTTTTTTTTTTTTCTCTTTTATTCCCTCC
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CCAGAAAACGAGTCTTAGTCATCTCATTCTCTCTGGCTTCACTTTTACCCTTAAGTGAGGAGACTATACCCGAGTTCC
ACCTAGCTTGCAAATTTCTATAATTCAATTATTCATCACCATTCTGAAATATACCATGTAATTATAGAAATTAAGTAA
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CCAAATCAAAACCAATGAGATACCATCTCACACAGTCAGAATGGCTGTGATTAAAGAGTCAAAAAACAACAGATGC

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CCAATACTAAAATTTGTGCCTTTGATAATATTTATATTATGAATAAAAAATATGCTCTTTTAACCATGTCCTCATCTATT
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Fig. 6. 108

[illegible]

Fig. 6.109

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AGACACAAGAGAGATAAGAGCTGATTGTTATGGCAAGGTCTCTGGCTAGGCAGAAAGTGAATGGGTGGAAGAGAACAGAT
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Fig. 6. (10)

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AATAAGATAGTAATTACCACTGACCCAGAAAAATACAAATAACCATCAGAGACTACTATGATGGTTTGTGTACTATGC
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Fig. 6.119

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Fig. 6. (2)

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ATTTTATCAAGGCGCTTTTCTGCATCTATTGAGATAATCATGAGGTTTTTGTGATTGGTTCTGTTTATGTGATGGGTTA
TGTTTAATGATTGTCATATGTTGAACAGCCTTGTATCCCAAGGATGAAGCTGACCTGATGTTGCGTGAAGCTTTTT
GATGTGCTGCTGGATTCTGTTTGGCAGTATTTTATTGAAGATTTTTGTCATAGATATTCATCAGGGATATCGGCTGAAA
TTTTTCTGTTGTCTCTGCCAGGCTTTGGCTTCAGGATGATACTGGCTTCATAAATGAGTTAGGGAGGACTCCCTCT

Fig. 6. [22]

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TTTTCTATTGATTGGAGTAGTTTCAGAAGGAATGGTACCAGCTCCTCTTTGTACCTCTGGTAGAATTGAGTTGTGAATC
TGTCTGGTCTCGGGCCTTTTATGATTGGTAAGCTATTAACTGCGCTCAATTGAGAACTTTGTATTGGTCTATTTAGG
GATTCAATTTCTCTCGATTAGTCTTGAAAGGGTGTATGTGTCCAGGAATTTATCCATTTCTCTAGATTTTCTAGTT
TATTTGCATAGAGCTGTTTCATAGTATACTCTGATAGTAATTTGTATTTCTGCGGGATCAGTGTGATATCCCTTTATC
ATTTTTTATTGTGTCTATTTGATTCTTCTCTCTTTCTCTTTATTAGTCTGGCTAGTGGTCTATCTATTTTGTAAATC
TTTTTTAAAAAAAACAGCTCCTGGATTGATTGATTTTGAAGGGTTTTTCATGCTCTCTATCTCCTTCAATTTCTGCTC
TGGTCTTAGTTGTTTCTTGTCTTCTGTTAGCTTTTGAATTTGTTTGTCTTGTCTTCTTTAGTTCTTTAAATTTGAGCGT
TAGGGTGTCTCGAATTTAGATCATTCTGCTTTCTCTGTTGGGCATTGTTGCTCTATAAATTTCCCTGTAAACAGTACTTTA
GCTGTGTCTTAGAGATTCTGGTACATTGTATCTTTGTTCTCACTGGTTTCAAAGAACTTATTTATTTCTACCTTAATTT
CATTATTTACCCAGTAGTCATTGAGGAGCAGGTTATTGAGTTTCCATGTAGTTGTGTGGTTTTGAGTGAGTTTCTTAAT
CCTGAGTTCTAATTTGCTCTGTGGTCTGAGAGACTGTTGTTATGATTCTGTTCTTTTGCATTTGCTGAGGAGTGT
TACTTCCAATTTATGTGGTCAATTTTGAATCAGTGTGACAAGGTGCTAAGAAGAATGTATATTCTGTTGATTTTGGGTG
GAGAGTCTGTAGATGCCTATTAAGTCTGCTTGGTCCAGAGCTGAGATCAAGTCTGAATATCCTTGTAAATTTCTGT
CTCAGTGTAGTGTCTAATATTGACAGTGGGGTGTAAAGTCTCCCAATATTATTGTGTGGGAGTCTAAAGTCTCTTTG
TAGTTCTCTACAACTTGTCTTATGAATCTGAGTACTCCTGTATTGGGTACAAATATATTAGGATAGTTAGCTCTTCT
TGTTGCGTTGATCCCTTTACCATTTATGTAATGCCCTTCTTGTCTTTTGTGATCTTTGTTGGTTTAAAGTCTGTTTTA
TCAGAAGTTAGGATGGCAACCTCTGCTTTTTTATTTGCTTTCCATTTGCTTGGTAAATATCTCTCCACCCCTTTGTTTT
GAGCCTATGTGTGTTGTTGACGCTGAGATTGGTCTCTGAATACAGCACAACAATGGATCTGCTCTTTATCCAATTT
GCCAGTCTGTGCTTTTAAATGGGGCATTATCCCATTTACATTTAAAGTTAATATTGTTATGTGTGAATTTGATCCTG
TCATTATGATGCTAGCTGGTTATTTTGGCCATTAGTTGATGTCAGTTTCTCATAGTGTGATGGTCTTTACAGTTTGGT
ATGTTTTTGCAGTGGCTGTTACTGGTTGTTCTTTCCAAATTTAGTGCTTTCTTACAGGAGCTTTGTTAAGCAGGCTG
GTGGTGACAAAATCTCTCAGGATTTGGGTGCTGTGTAAGGATTTTATTTCTCTTTCACGTTTGAAGCTTAGTTTGGCTG
GATATGAAATCTGGGTGAAAATCTTTTCTGGGGGAGGAGCCAAAGATGGCCGAATAGGAACAGCTCTGGTCTACAA
CTCCAGTGAGAGCGTCACAGAAGACGGGTGATTTCTGCATTTCCATCTGAGGTACTGGGTTTCATCTCACTAGGGAGTG
CCAGACAGTGGGCGCAGGTGAGTGGGTGTCATGCACCATGCGCGAGCCGAAGCAGGGTGAGGCATTGCCTCACTCGGGAA
GCACAAGGGGTGAGGAGTTCCCTTTCTAGTCAAAGAAAGGGGTGACAGACAGCACCGGGAAAATGGGTCACTCCCA
CCTGAATACTGCGCTTTTCTGACGGCCTTAAAAACGGCACCAGGAGATTATCTGTCACCTGGCTTGGAGGGTCTTA
CGCCAACAGAGTCTCGCTGATTGCTAGCACAGCAGTCTGAGATCAAAGTCAAGGTGGCAGCGAGGCTGGGGGAGGGG
GCCCACTTGGCCAGGCTTGTCTAGCTAAACAAAGCAGGAGGCTCAAAGTGGGTGGAGCCCAACCAAGCTCAAG
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CACCTCACACGGCTGGGTACTCCAACAGACCTGACGCTGAGGGTCTGTCTGTTAGAAGGAAAACCTAACAAACAGAAAG
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GGAACAGAGCTGGATGGAGAAATGACTTTGATGAGCTGAGAGAAGAGGCTTCAAGACAAATCAAATTACGCTGAGGTACTG
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CAGAGAAGTGCTTAAAGGAGCTGATGGAGCTGAAAACCAAGGCTCGAGAACTACATGAAGAATGCAGAAGCCTCAGGAG
CTGATGCAATCAACTGGAAGAAAAGGTATCAGCGATGGAAGATGAATGAATGAATGAAGTGAAGGGAAGTTTAGA
GAAAAAGAAATAAAAGAAAAGGGCAAACCTTCAAGAAATATGGGACTATGTGAAAAGACCAATCTATGTCTGATTG
GTGTACCTGAAAGTGACGGGGGAGAATGGGACCAAGTTGGAACCACTCTGACAGGATATTATCCAGGAGAACTCCCCAA
TGTAGCAAGGCAGGCCAAAATTCAGATTGAGGAAATCAGAGAATGCCAAAAGATACTCTCAGAGAAGAGCACTCCCA
AGACACATAATTGTGAGATTCAACAAAGTTGAAATGAAGTGAAGAAAATGTTAAGGGCAGCCAGAGAGAAAGGTGCGGTTA
CCCTCAAAGGGAAGCCCATCAGACTAACAGCGGATCTCTCAGCAGAACTCTACAAGCCAGAAGAGAGTGGGGGCCAAT
ATTCAACATTCTTAAAGAAAAGAAATTTCAACCCAGAATTTTATATCCAGCCAACTAAGCTTCATAAGTGAAGGAGAA
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CACTAAACATGGAGAGGAACAAATGGTACCAGCCACTGCAAAATCATGCCAAATGTAAAGACCATCGAGACTAGGAAG
AACTGCTATCGATTAAAGGAGCAAAATAGCCAGCTAACATCGTAATGACAGGACCAAAATTCACACATAACAATATTAAT
TTAAATGTAAATGAGCTAAATGCTCCAATTAAGACACAGACTGGCAAAATTTGGATACAGAGTCAAGACCCATCAGTGT
GCTGTAATCAGGAAAACCATCTCAGCTGACAGACACACATAGGCTCAAATTAAGGATGGAGGAAGATCTACCAAGC
AAATGGAAAACAAAAAAGGCGAGGGTTGCAATCCTAGTCTCTGATAAAACAGACTTTAAACCAACAAAGATCAAAAGA
GACAAAGAAGGCCATTACATAATGGTAAAGGGATCAATTCAACAAGAAGAGCTAACTATCTTAATATATATGCACCCA
ATACAGGAGCACCCAGATGCATAAAGCAAGTCCCTGAGAGACCTACAAGAGACTTAGACTCCCACACATTAATAATGGG
AGACTTTAACACCCCACTGTCAACATTAGACAGAGCAACGAGACACAAAGTCAACAAGGATACCCCTGGAATTTGAATCA
GCTCTGCACCAAGCAGACCTAATAGACATCTACAGAACTCTCCACCCCAATCAACAGAATATACATTTTTTTTTCAGCAC
CACACCACACCTATTCCAAAATGACCACATACTTGAAGTAAGTCTCTCAGCAAAATGTAAACAGAAATATAAC
AAACTATCTCTCAGAGCAAGTGCATCAAACTAGAACTCAGAACTTGAAGTAAAGTCTCAATTCAAAACCGCTCAACTATGG
AAACTGAACAACTGCTCTGAATGACTACTGGGTACATAACGAAATGAAGGCAGAAATAAGATGTTCTTTGAAACCA
ATGAGAACAAAGACACAGCATACCAGAATCTCTGGGACGCATTCAAAGCAGTGTGTAGAGGGAATTTATAGCACTAAA
TGCCCAAGAGAAAGCAGGAAAGATCTAAATGGACACCTAACATCACAATTAAGAAGACTAGAAAAGCAAGAGCAA

Fig. 6.125

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ACACATTCAAAGCTAGCAGAAGGCAAGAAATAACTAAAATCAGAGCAGAACTGAAGGAAATAGTGACACAAAAACCC
TTCAAAAAATTAATGAATCCAGGAGCTGGTTTTTTTGAAGGATCAACAAAATTGATAAACCGTAGCAAGACTAATAAA
GAAAAAAGAGAGAAGAATCAAATAGACGCAATAAAAAATGATAAAGGGGATATCACCACCAATCCGACAGAAATACAA
ACTACCATCAGAGAATACTACAAACACCTCTACGCAATAAACTAGAAAACTAGAAAGAAATGGATAAAATTCCTGGACA
CACACACTCTCCCAAGACTAAACCAGGAAGAAGTTGAATCTCTGAATAGACCAATAACAGGCTCTGAAATTGAGGCAAT
AATTAATAGCTTACCAACCAAAAAGAGTCCAGGACCAGATGGATTACAGCCGAATTCTACCAGAGGTACAAGGAGGAG
CTGGTACCATTCTCTGAACTATTCCAATCAATAGAAAAAGAGGGAATCCTCTCTAACTCATTGTATGAGGCCAGCA
TCATCTGTGACAGCAAGCCAGGCAGAGACACAACCAAAAAAGAGAATTTTAGACCAATATCCTTGATGAACATTAGGATGC
AAAAATCCTCAATAAAATACTGGCAAACCAATCCAGCAGCACATCAAAAAGCTTATCCACCATGATCAAGTGGGCCTC
ATCCCTGGGATGCAAGGCTGGTTCAATATACGCAATCAATAAATGTAATCCAGCATATAAACAGAACCAAGACAAAA
ACCACATGATTATCTCAATAGATGCAGAAAAGGCCTTTGACAAAATTCACAATGCTTCATGCTAAAACTCTCAATAA
ATTAGGTATTGATGGGATGTATTTCAAAATAATAAGAGCTATCTATGACAAACCCACAGCCCAATATCATACTGAATGGG
CAAAAATCGGAAGCATTCCCTTTGAAAATGGGCACAAGACAGGGATGCCCTTCTCTCACCCTCTCTATTCAACATAGTGT
TGGAAGTTCTGACCAGAGCAATTAGGCAGGAGAAGGAAATAAAGGGTATTCAATTAGGAAAAGAGGAAGTCAAATTGTC
CCTGTTTGACAGCAAGCATGATTGTATATCTAGAAAACCCCAATGTCTCAGCCCAAAATCTCTTAAGCTGATGAAGCAAC
TTCAGCAAAATCTCAGGATACAAAATCAATGTACAAAATCACAAGCGTTCTTATACACCAACAACAGACAAAACAGAGA
GCCAAATCATGAGTGAACCTACCATTACAATTGCTTCAAAGAGAATAAAATACCTAGGAATCCAATTACAAGGGATG
TGAAGGACCTCTCAAGGAGAACTACAAACCACTGCTCAAGGAAATAAAGAGGATACAAACAAATGGAAGAATATTC
ATGCTCATGGGTAGGAAGAATCAATATCGTGAAAATGGCCATACTGCCCAAGGTAATTTAAGATTCAAGTGGCCATCCCC
ATCAAGCTACCAATGACTTTCTTCACAGAATTGGAAAAAACTACTTTAAAGCTCATATGGAAACATAAAAGAGCCCC
CATCACCAGTCAATCCTAAGCCAAAAGAACAAAGCTGGAGGCATCACACTACTTGACTTCAAACATACTACAAGGCT
ACAGTAATGAAAACAGCATGGTACTGGTACCAAAACAGACATATAGATCAATGGAACAGAACAGAGCCCTCAGAAATAA
TGCTGCATATCTACAATCTCTGATCTTTGTCAAACCTGAGAAAAACAAGCAATGGGGAGAGGATTCCCTATTTAATAA
ATGGTGCTGGGAAAATGGCTAGCCATATGTAGAAAGCTGAACTGGATCCCTTCTTACACCTTATACAAAAATCAAT
TCAAGATGGATTAAAGACTTAAATGTAGACCTAAACCATAAAAACCCCTAGGAGAAAACCTAGGCATTACCATTGAGG
ACATAGGCATGGGCAAGGACTTCATGTCTAAAACACCAAAAGCCATGGCAACCAAGCCAAAATTGACAAATAGGATCT
AATTAACCTAAAGAGCTTCTGCACAGCAAAAGAACTACCATCAGAGTGAACAGGCAACCTACAAAATGGGAGAAAATT
TTCACAACCTACTCATCTGACAAAGGGCTAATATCCAGAATCTACAATGAACTCAACAAAATTACAAGAAAAAACAA
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AAAATGCTCACCATCACTGGCCATCAGAGAAATGCAATCAAAACCACAATGAGATACCATCTCACACAGTTAGAATG
GTGATCATTAAGAGTCAAGAAACACAGGTGCTGGAGAGGATGTGGATAAATAGGAACACTTTACACTGTTGGTGGG
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GTGGCATTTATCAATAGCAAGACTTGGAAACCAACCCAAATGTCCATCAATGATAGACTGGATTAAGAAAATGTGGC
ACATATACACCATGCAATACTATGCGGCCATAAAACATGATGAGTTCATGTCCTTTGTAGGGACATGGATGAAATTGGA
AATCATCATTCTCAGTAACTATCGCAAGAACAAAAACCAACACCGCATATTCTCACTCATAGGTGGGAAATGGAACA
ATGAGAACACATGGACACAGGAAGGGGAATATCACACTCTGGGGACTATTGTGGGGTGGGGGTAGGGGGAGGGATAGC
AATGGGAGATATAGCTAATGCTAGATGACGAGTTAGTGGGTGCAGCACACCAGCATGGCACATGTATACATATGTAAT
AACCTGCGCATGTGTCACATGTACCCATAAATCTAAAGTATAATATAAAAAAAGGAAAACTAGAGTATAAATGAAG
TGTCAAAGATGTTAAGAAACAGGATCTTTTAAAAAATGTTTTTAAATTATTATGGGTACATAAATAGTGTATATCTA
TGGGATACATGTGAAATTTTGATACATACAAATATAAATCTTATTGGGGCAATTGGGGTGTCCATCACCTCAGGCA
TTTATCATGTCTTGTATTAGAAACAGTCCAATTTCTCTCTTTTAGCTATTGAAAATATACAATAAATTTATTGTTGAGT
ATAAAAAAAGTCCACACCTAGTCATATTATGTTCAAACTACAGAAAACTAAAGACAACCCAGAAAAACATGAAAGAAGC
TACAGAAGAGAAGAAATTTACCTAAGGAGGGACAAGGATAAGAATTACATCAGAAATCTCATTGGCAACCATGCAAGAA
AGAAGAAGGTAGCGTGAAATATTTAAAGTGTTTAAAGGATAAACTAGAATTCTGGATACAGTGAACCTATCCTTCAAA
AGCAAAGAAGAAATGCTTTCTCAGACAAAGGCTGAATCTGTACCAGTAGACCTGCCCTTGAATAAATGTTGAAAGAAA
TTTTTCGGTCAGAAATGAAATGACATAGGTCAAAAACCTGAAATCTACGTAAAGAAAAGAGGCTTTCAAGAAAGGAACA
AATGAGCATAAAAATAAATCTTTAATATTCCTTAACCTTAAAAAAGAAATTCCTTCTTTAAGAGTGGCCC
CCTCTCTTTCTGTCTTGTAGGTTCTGTCAGAGAGATCCACTGTTAGTCTGATGTGCTTCCCTTTGTGAGTAACTGA
GCTTTCTCTCTGGCTGTCTTACATTTTTCCTCATTTTCGACCTTGGTGAATCTGATGATTATGTCTTTGGGGTTG
CTCTTCTCGAGGAGTATCTTTGTGGTGTCTCTGTATTTCTGAAATTTGAATGTTGGCCTGTCTTGTCTGGGTTGGGGAT
ATTCTACTGGATAATATCCTGAAGGGTGTTTTCCAACCTGGTTCCATCTCCCATCACTTTGAGGTACACCAATCAAAC
GTAAGTTTGTGTTTTTACATAGTCCCATATTTCTGGAGGCTTTGTTCAATCCCTTTCATTCTTTTTTCTCTAATCTT
GTCTTACCCTTTCTTTTCAATTAATGATCTTCAATCTCTGATATCCTTTCTTCTGCTTGATCAATTTGGCTATTGATA
CTTGTGTATGCTTCAGGAAGTTCTTGTGCTGGGTTTTTCACTCCATCAGGTCGTTTATATTCTTCTCTAACTGATTA
TTCTAGTTAGCAATTCCTCTCACCTTTTTTCACTGTTCTTAGCTTCTCTGCAATGGGTTAGAACATCGCTTCTTAGCTC
GGAGCCATTTGTGTTTACCCACATTTTGAAGTCTACTTCTGTCATTTGTCAAATTCATTCTCATCCATCCAGTTTGTTC
CTTGTGTTGAGGAGTTGTGATCCTTTGGAGAAGAGGCATTCTGATTTTTGTAATTTAAGACTTTTTGCACTGGTTCC
TCCCCATCTTCATGGATTTATCTACCTTTGGTCTTTGATGTTGGTGACCCCTGGATGGGGTTTCTGACTGGACATCCTT
TTTGTGACGTTGATGCTACTCCTTTCTGTTTGTAGTTTTCTTCTAACAGTCAGGCCTCTCTGCTGCAGTCTGCTG

Fig. 6 (129)

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GAGTTTGTGATGTCCACTCCAGACCCTCTTTGCGTGGGTATCACCAGCAGAGGCTGTAGAACAGCAAAGATTGCTGC
CTGTTCTTTCTCTGGAAGGTTTTTCCAGAGGGGCCACCAGATGCCAGCTGGATCTCTCTGTATGAGGTGTCTG
TCGACCCTGTGGAAGTATCTCCAGTCAGGAGGCACGGGGGTGAGGGACCCATTTGAGGAAGCATTGTGTCCCTTA
GCAGAGCTCAAGCACTGCGCTGGGAGATCCACTGCTCTCTTCAGAGCCAGCAGGCAGGAATGTTGTCTGCTGAAGCAG
CGCCTACAGGCACCTCTTCCCCCAGGTGCTCTGTCCCAGGGAGATGGGAATTTTATCTATAAGCCCCCTGACTGGGGCTG
CTGCCCTTCTTTCAGAAGTGCCCTGCCAGAGAGGAGGAATCTAGGAAGGCAGTCTGGCTACAGTGGCTTTGTGGAGCT
GAGCCCAGTTTGAACCTCTGCTGGTGGCTTTGTTTACACTGTGAGGAGAAAACCGCCTACTGAAGCCTCAGTAATAGCAGA
CACCCCTCCCCCTACCAAGCTCAAGTGTCCAGGTCCACTTCAGACTGCTGTGCTGGCAACAAGAAATTTAAACTAGTG
GATCTTAGCTGTGCTGGGCTCCACAGGGGTGGGATCCGCTGAGCTAGACCACCTGGCTCCTTGGTTTCAGCCCCCTTTCC
ACAGTTGTGAATGGTTCTGTCTCACTGGCCTTCCAGGTGCCACTGTGGTATGAAAAAATCCTGCAGCTAGC
TCGGTGTCTGCCAAACAGCCACCAGTTGTGTGCTTAAACCCAGGATCCTGGTGGTGTAGGAACCCAAGGGAAATCTC
CTGGTCTGCAGGTTGCAAAGACTGTGGGAAATGTGTAGTATCTGGGCCGGAATGCACCATTCTCTACAGCACAGTCCCT
CATGGCTTCCCTTGGCTAGAGGAGGGAGTTCCCCAACCCCTGAACTTCCAGGTGAGTTGATGCCAACCCCTGCTTCA
GCTCACAGCCGCTGGTCTGTACCCACTGTCTAACCTGCCAATGAGATGAGCTGGGTATCTCCGTAGAAATGCAGAA
ATCACCTGCCCTTCTGCATTGGTCTCGCTGGGAGCTGCAGACCGGAGCTGTTTCTATTCCGCCATCTTGCCAGCCAGCCA
CTGTAATTCACCTATTTTTCAAATGTTACCATTAGAAGTGTATACCATATTACAAGTTTCAAACACTATCTGCCAGGGC
ACAAAAGAGTTCTTAGTTGCCTTAAAGAATTAGCTATTGTTTCATCTGTGTGGCTTCGTCTCAGAGGGTGATTGAAA
CATGACAAGAAGTGATTTTTTTTTTCTCAAGAGAAATGGAGGCTTTGGACATTAGTGTCTAGCAATGTCTGTAGAACA
TACATAGAGAATTCTATGAAGAAATCAGCCAAGCCGTGACTATCGACCAAAATTTTCAACTTTCAACAATGAGATGAAT
ATACTATCTATATCTAATAATCCAAAGCTTCAATTAAGAATATGCTTCTCAAATAAAAAATTTGGAGCAGTTTG
TGATTTAAATGATGGAATTTTTTATAAGAACTAATTAATGCGCAGTTAAATATATAAACTGAAGTTACTAATATGAACTCAGT
GAGCCTTTTCATGAGCTTTTATTTTAAACCAGCTAAAATACTAAATACTTTTATTTTAAATCAGCTAAAGCATTCAACTTA
TAGTGAGGAGAGTTTTGTTTTCAACACAGTTACGAAAGAATTCGGGCTCACTTAATGTAAATAAAAAATCTTAAAGAAG
AAACCATTGTTTAAATGTTTACCTCATTGAGTTTTTCAGTAAATGGAACAAGAATTAACATTTAGGGTAATAATAGTTT
ATGCTCTGTTTTTAATTATCGGACAGAATACTTAGGGAATGGACAGAGGCAGTAAGAAAAATAGTTTATTATATATAA
TGTATCATTAGTTTTGAATGTGCATACACCTAATATTAAGTGTATAGTACACAATTTGGTCATTTTTATTTTTAAAA
TATTACCTCATATCAATAATCCTAATTTAAATGGTTAAGAACATGGAAATAATTTCCATGAAGTATGCATTTCTGAGTA
ATGGTTGTATATAACCAAAATGAAAGCTAATTAATTCATTGGTGAAGTTATAGTGAGATAAAGCACAGACTGTAGAC
ATATACAACATTAAATTAGGACAAATGTTATTCTACATCTACAGGTGAATTTCCACCCAACTGGAGGCTCATCAGCATT
TACCTTTTATTCAAAGGGGAAATTTGGTGAGAGAGTAGAGGCAGATAATCACAGCATCCCACTCTACTAGAAAAGCAAGC
CACAGCTCCCATCCTGCTGGTGATGTGACAGCACTGGTCTTTCTACACAGCAGCACACTAGTACCAAAAAAGAGGCTTT
GCTTTTTCTGTGTGATGAGCTGTAAACCTTCATATTAGAAAACTCAGAAAAAGAAATTTTGCTTAGACGCTAATCAAATA
CAAAAATTTGGCTGATGGAACTACACATAGATAAATTAGTCCAATATTCTTCTACTTGTGAAATTTAAATAAATTCA
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AACCTTAGCACAAATAGCAGAATTTCTGTATGGTGGCTATTACAATTTTACCAGTGAAGGACATCTATGGGTCTTT
GAAAAGCTAGGAAACATCTTGAATATCAGAAATTTGAATGAATACTACTTGGTGTAGATTAACTAAAGACATGGGAT
TATATGTTCTCAAAGCCACAGATGTAGTTGGGTCTAAGAACCTTGTCTATATATCTATATTTTTTATTTTTTTTATT
TTTTTTCTTTTATTATTATTATAGTTTAAAGTTTGGGTACATGTGCACAATGTGCAGGTTAGTTACATGTGTATACAT
GTGCCATGCTGGTGTCTGCACCCATTAACTTGTCTATTAGCATTAGGTATATCTCTAAAGCTATCCCTCCCCCTCC
CACCACCCCAACAGTCCCAAGTGTGTGATGTTCCCTTCCCTGTGCTCATGTCTCATTTGTTTCAATCCCCTAT
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CCTACAAAGGACATGAACCTATTTTTTATGGCTGCATAGTATTGCATGGTGTATATGTGCCACATTTTCTTAATCCAG
TCTATCATTCTTGGACATTTGGGTGGTTCCAAGTCTTTGCTATTGTGAATAGTGCCACAATAAAACATACATGTGCAT
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AAATTTGTTTAAAGTTCAATGTAGATTCTGGATATTAGCCCTTTGTGATGATGAGTGGTGGCACAATTTTCTCCATTT
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GAACAAAGCTGGAGGCATCACACTACCTGACTTCAAACATACTACAAGGCTACAGTAACCAAAACAGCATGGTACTGG
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AGACCTAAAACCGTAAAAACCTAGAAAGAAACCTAGACATTACCATTACAGGACATAGGCATGGCAAGGACTTCAATGT
CTAAAACACCAAAAGCAATGGCAACCAAGCCAAAATTGACAAATAGGATCTAATTAACTAAAGAGCTTCTGCACAGC
AAAAGAACTACCATCAGAGTGAACAGGCAACCTACAAAATGGGAGAAAATTTTCGCGACCTACTCATCTATATTTTTT
AATGATTTAAACAGCAAGAAAGACCATGGAGCCACCTAAATTTTTTCAAATCTGCAAAATGAAGGTGATATAAATAT

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GTCACCTTAGACAATATTTTCATTTTTGATATAAAATTTTTATTTTTATCAGTTTGATAATATGCATAGGACTAAAAAATG
CAGTTGTCTTAAATATTTTAGGGTTGCTTAGGAAATCACTTTAAAAATAAAAGTGTGAGAATAAAAGTTGTCTGCTT
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GCTTCAGCAAAAACCCACCAGGGCAGAGATCACTCTCCCTCTTGTGTTGCTTTGAATTTTTATGACTTAGCACAGTGAT
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TATGAACCTTAAATCTTTTTGTAAAGATCTAGTACCAATAAGATAAAAAAATGCTCATCTTTTTTCCATTGAAATGT
TATGTTCAAATGAGCTTTGTTTCTATACTTTATACATCAATTAAGCTGAATTCATAGGTGCTAAGCATTTTAAACATATT
GTATTGCATGTAATATCCCTAATGCCCAAACCTTCAAGATTATATAATGGCTTACTCTCTCCCTGTCCCTACCACCA
GATAGTGTTATCCACATACATTCTCATCTAGTTTTGTTCTGTGATGAAAAACCATATGGGTATCCTATTCTTATGTGAA
TTAACCTGGCATGCAGGTAAGTGAAGTGCATGTGATCTGGTCTCTGCTGACATATCACAATGGGCCCCCTCCTTGCAT
GGAGGTAGCGTTTTTTTAAAGACAAAATGTTTTAAATAGAACACATTTTCAAGATTTTATATGTATTTTGTGT
TTTTCTCCCTCTACCCCTTTCCAAAATTATGAATTAATCTAGGACCAATTTATAGACAAAGCAAGTTTAGTCCGAG
TAAACCAATGGACCTGAAGTGTCCAAGTCAGAGTTTTATTTGCAGAATTTAATTCATCCACAGAGATAGGCAACCCA
GGCCTGTGAGGACACGAGGGTAAGTACAGCAACAAAGTGCCGTAGTCAGGCTGTGTTTGTCTTTTTGGTAAGAGGACAAC
ATTGACTTCAGTGTGAGGGCATAAAGGAGACTCAGGACTTATTAATTTTTTCCCATAAATCTGTGAACCTTTGTGAAT
TCCCTAATATTTCTTTAACAAGAGTTCCGGAGACATGAGTTTATGTGCTTCTTGGATATATTCACGGGAGTTTGCAGAG
AAAGTTGAATAAAATATTAGGTTAATGGCCTGTGTAAATTCACCAACACCTTTTCACTATCTCATCAATCATCTCTGTT
GAGTTATTGTGATTCAGTCTGCTGATGAGCTCACACCTTTTTCTTGATACAGGAATTTATGTATACAGGGGACTGG
ATTTTAAACAAAACGTATTTCTTGAATAAATCTGAACATGAGTTTGGTGGTCTTACACTATTATGTGTGTGTAGCT
GTACAAGTGTGTCTGCATGAGCTTTAGGACATTATTTGAGATATTTTAAGCTATGTGTACCTCATGAACCTGTAGCTGA
TTTTCTTAGTTCTTTTTAAATATTTTCTCAGAAAACCAACAGTAAATCTATCAGGTTTACATGAATACACTCATTTG
TGTATATCAACCCAAAATGAATATGATCTTCCAGGTAATGATGAAGGATGATAACTATAATATTTCCAGCCAACTTT
ATTTTGAACATCACTCAGTGTTCACATGTTTAGTGCTGTTAAATCTATATGTCTAAGCAAACGTGTGAAGAGCATA
ATTAATTTATTTGGTTGTTGTGTATCTTTTAAATCACAGTTTGAATCTGCTGGGAATGTTATAGTGGCACTAGTAGCA
AAGGAATGGCAAGGATGTTAAGACTTTCTCATGCTAAGACCCAGCTTGGTATTGAGTTTTTAGGAGGGGGCCGCATGA
CATCTCTAATTTGCTTTGGCAATCCAATTATTGAGACAAGTAAGTGAAGCCCATCGGGTGCTTCCCAAGGAATTAACCTGG
TCATTTGATATAATGTTGCACTCTCCCTATTTGGGAGGAGGGGTGGCCACCCTCACCTCTGCCATTGAAGATTAAACCCA
CTACAAATTTTCAAAAATATAATATCTAATTTATTACACAGACTATCATATGGGTTTCATAATCCTTAAGTATCTCTAA
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AGCAGAAGTAGAAATGGTGTGAAGCCGCAAGCCAAAGGCATGCAATGCTGAAAGCCTCTAGAAGCTGGAAGAAGAAAGG
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Fig. 6. 128!

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TCTGCTTCTAAGGTTGTTTCAGCCAGCCTTATAATGCAACTAGTGTGAGAAGCAGTGACACAAAAAGAATAGAGCCAA
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TCACCTTGACCTAGAGCTGAGTTCAGGTCCTGAATATCCTTGTTAATTTTCTGTCTTGATTATCTGCCTAATATTGACAG
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Fig. 6.130

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GCTGGTCTGGGCTGGATGGTCCAAGAAAGTTTCAGTCATATACCTGGCACCTCTGAGCTTTTCCATATGGCCTCTATGT
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Fig. 6.13j

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Fig. 6.41

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TTATCATGTCTCATAGTTAAACCAGATAGTTTGGTAGATTCTTTATCACGTGTGTTTCTAATACAACCAGGAAAACCTA
TCGTATATTCTTTTTTTTTTTTTTTTTTTTGTGAGACGGAGTCTTGCTTTGTGCGCCAGGCTGGAGTGCGGTGGCAGC
ATCTCGGCTTACTGCAAGCTCCGCTCCAGGTTACGGCTTCTCTCTCAACCTCCTGAGTAGCTGGGAATACAG
GCTTCCGCCACCATGCCCCGCTAATTATTTTTGTTATTTTAGTAGCGACGGGGTTTACCCTGTATAGCCAGGATGGTCT
CGATTTCCCTGACCTCGTGATCCACCCGCTTGGCTCCCAAAGTGCTGGGATTACAGGCATGAGCCACCGCGCCAGCC
TATCATATATTCTTACATTCTCTCTCACACAAATGTGCATGCGCACACACACATGCACACATATACATCATTTCTGAC
ACTGCCTTAACTTTCAGAATACAAGGTTGAGTCTCTGCCACATACTAGCAGTATAATCCTGGATGAGTTACTTAACT
TACTTTGCTTCAGTTTGAGGATGAAATGAGAGAATATGTGTAAGACATCTGGCACACATAGTGAGCATTCAATAAATGT
TAACTATAAGTAGGTGACCACTTGGGGCCCAAGGAGATTGTAACCTTGCTTAAAGGTCATTAAAGGTAGTTCTGTTGCTAAGT
CAGAATTAGAATCTACATAATGATTCTCAATCAACCTCTTTTATACTAAGTTATCTCACTAATTTGGCAGCTCTTTCCT
GTGTTAACTTTCTGTATGATAAGGGTTTGCCTTCCCAAGTAAGTTGTAAGCATCTCAAAGGTTAAAGAGCTTGTGAGT
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TCCTCAGAGAAAATCATACAATTTTCTACTATATTTCTGCAGTATGAAGAAAATAATTTAATATACCTATGCTTTGGTT
TCTCCATATACAGAACTTATATATGGCATTACTCAATCACCAGAAAGATTCCATTATAATTTAGCTTATTGTTATGTA
TTCAAGGCAATAACCCAGGCCACAATATGATCCCAAGAAATAACAAGTATCCATAGGGGAAAACCTGGTAGAATACTCAA
GAGTGGGAATTTATTTTAGCTTTGGATGACTTTTATATAAAGGAGCTCCACTCACAATACATTAGTGGCACTTAACT
ACACTCGCTAGTAAGCTAATATTTGAGGTCCATTTTTTACATCTCTTTGAGAGTATATCAGTATATCAAGCATTACAAA
TTACTACCTTGATTGCTTTGGAGCTACTCTTTTTTTGAGACAGAGTCTTGCTCTGTCAACCAGGCTGGAGTGCACTGG
CACCATCTCAGCTCACTGCAACCTCCACCTCTTGGGTTCAAGTGATTCTCTGCTCAGCCTCTGAGTAGCTGGGATT

Fig. 6. [45]

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ATAGGCGTGCACCACCATGCTCAGCTAATTTTGTATTTTGTAGAGACAGGGTGTCACTATGTTGGTCAGGCTGTTTCT
TTGAACTTCTGACCTTGTGATCCACCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCATGGGCCACCGTGCTGGC
CCTTAAGAGCCAACCTAGCATATCCTTGTAATCAGTTGGCTAAACAAGCTTCTCTTAAATATGCTTTGAAAAATAT
AAATTCCTCTCTATCAGTATCTACATTTCACTTTGAAGATCTTATCGGCTTTCAGCAGCAGGTGTGCATATCCTACAAG
TTTCAGTTTTCTCTTGATGCTCAGGATGAACTAAACATCTAGACAAGGGAGCATCTGGGAAGTCTGACTAACTGGACTA
CTTGGACTGGTTTGTCTCACAAAATGTAAGACTTACAATCTGTAGGCTGTTACCTAGCTCCTGAGAGCTACCTCCCCC
GGGCCGCCCACACTAGATTTTGTAGCTCTGAGCTGCATACCCAGGAATTCTCTGTTTCCGTGAATCAGGAAGGATATGG
CAAGTTAAGAGAGAAGGAAAGAACCTGCTGAGCCTTGCCCTTCCAAGGGATATCAGGAAGTTACTCAAAGACGGCAAAG
TGCTCTCTACACAAAAGTGCTGGAAGAACTGCAGTTTATATCCCTTTAGGAAAGGAACTTTAAGATGATTTGGAATA
GCCAAATGTAATCACATAACAACCAATTTGAAGTTTGTGGCATGCAACACTCCTTTAGGAATTTCTGGTTTCCAGCT
TGGTCTCATTGGCAACAAATAGAATTTCTTAAGCCTTATTAGGAGCCTGACATTTTGTGTTGAGAATCAAACCTTTTCTG
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CGGTTATACAAGGTAAAAATGAGTTACTGGCCCTTCAGGATGGGTATCATGATAGCTCATTAAATTTAAATAACTCCTAA
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AGCAAATGGCTTCTATAGTTGAATAATCTTTCAATAAAGTTAGAAAAATGGGTGTCTTCTCAATGCATAGCATGTTG
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GCTGATCTCTTATTTCTTGTCAACCTAAGGTACAGTGGGTATTAAACTCTTTTAGCTTCTGAAAGACAACTAATTCCC
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TTGTCTGTGGTAGGTTGCTTGGCCACCACTTGGCCAGTATCATTATCTGTGACTTGAGGCAAATGGTAAGGACAGCTTG
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GCCCCAAGCCAGTATGGGACCACTCTACAGTATGGAAGGCAGCTCAGACATAGGTGAGTTGTTCCAGGAGACCACACA
TTATGATCTGTTGAGCTGTTTCATCATTTTCACTTGAAAATGAGGGGTGGCTGTTTTTAGGCTGTGAGCCTCAGATTTGA
ATTCTATAGTGATTAGGTGGTGAATGGCTGCTCTCTGCAGACTGCTCAGGGGCTGTTAGAGCTACACAATTGCCCCAA
AATCAACAGGAGCAAATAGCCTCACCAAAACCAGCCTTGAGTCATTCTTTAAGTGAGGGTGATTAGGAGGGTCAGCTAT
TTTTAAGAAAGAAATTCATAATCAGTCTGTCCAGAGACTCAAAATATTATTCATGTCATTTTCCAATGTCACCTTTC
CTCACCATCACTTTGTTGGCATTAGGCTTTGAGACATGGTCTCACCAGCAAGATGGTTATTTTTATGTCTTATCAAGG
TCTCTAACAGGTCCTATGACACGCAAAATGTTAGAAAGGAGAATAAAGCAACAATAGTAAGCAATAGTACAGTGGGA
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CAGAGGTGAAATCTTTAGACAGATGAGTCTCAAAGAGGAAATATGCAGAGCCAAATGGCTACAAATGGTACTGAGGA
TTGAAACCTAACTATAAGTGAAGTCTCTTCCCTAAATGATACACTCAGACTTAAACAATAAGCTAATAAGGTGGTTCA
ATAAAGAAATGTGGGTCTGATGGAAGAGTAGAGAGTTTAGAAGGTGAGTAAGAGATATCTATGATATTAGCTTGGGTG
TGTTTTTGGTATTGATGCCATTGTGTTGGGAGTACAGCAGAGGACAAGAACTCCTTTAGCAACTTGGCCTGCAGTCA
AATAGGCCAACATTGGAATCTGAGCTCCACTGCCTTCTTCACTGGGACTAATCACTTACCCTCTCAGCTCTCATCT
GTAAAAAAGGATAATAATAATAATGTTACCTTTGAAAATTAACACCTTCTCTCTGTACCTTTAACCTTT

Fig. 6.145

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CTCTCAACTAGATACTTTCCATGAGACTCAAATACATTCAAGTGTCTTCTGCATTAAAAACAAACAAAACAAAACCT
CACCTTCAACCACTTATTTCCCTATGTTAGGGTTTCTCTTGACGTTTGGGGCTAGATAATTCTTTACTTTGGGGCTGTT
CTGTGCGTTTGGGATATTTAGCAGCATTTTACCCATGAAAATATTGTGTCCCTCGCCCCACAAGTTGTGGCAATCA
AAGTTGTCTCTAGACATTGCCAAATATCTCCTGGAAGATGCCACCCCCACCCAGTTAGGAACCACTATTCTAATG
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CCTATCACAGCCGATCCTATTTCTAGTCTGTCTCTTGTTCCTATTTTACCAATAAAATCATTTTTAGTAAGAAGAAACAAT
AACCTGGATAGTTCTAAATGTAATGAATATTGCAATTCTTCTCTTCTTCTGATTCTCACAATTTGAAGTGTGTTCTTCT
CCTGGCTCCTCTGACACTATTCTCTCTCGGCTTGATCTGTGAAGCCTGGGTCTCCTGGAATAGCTGCAGCCTTCTCTCT
CACTTTCTTACCATATTCTCTCACTGTGTCCAGGACACTAGCTCAGACTTTTTCAAATGCTGATTAAAGGGCTTCCATG
CTGACAGAGGGCAAACCTCACAGAGCAAGCTTTTCTTAAGCTTTTGCTTATATCACATTTGCTAGCACTCTCTTGGCCA
ATGCCAGAGTCACTGGAAGAGCCTACTGAAGGCAGAAATACAGAGAGAGAAGACTTTTGGCATAACATCTTGAGGG
CTTCTTTAAACCACTCCCCAGTAAACTAGTGAAGAACTATGATCAAAACAAATAAAACTCCAAGCACTTAAATCCTCT
GGAAATGATCCTAAGGACAAATAGCAAATGAAGAAACATCTATTCAAGAACATTTATGAAAATTCAATAAGAAAGGCAA
GCCTGTGGTATTAAACCAAGACTGCTCCCTCTCACCCCTTCCAAGTTTCAAGGAGATGGAGCTTCCATTCCAGGCTGG
TGCAATCAAGAACACAGAGCTCTCTCCCTCCAGTGAAGTTTCTTCTGGAAGGAACAGAACTTCACTGTTTCTCTC
TCCTGGCCATAGTTACCCATTGCTAAGGCTAAGCTCTGGTGAATACAGTAGAGAGGTAGGGGCTTCTTCCCTGCCAAA
TCCCCCATCATTGAATGGAGGGGATACCTTAGGCACTGCATGCTAAGAATACAGAGGCTCATCATCTTGCCTGGCCT
CCTGAGGTGGGGGTTCCACACCAGGAGAGATAAATATAGAAGATATTAGAGTGTGCCACCTCCCACTAAGCTAAGCT
CCTAGAGTGGGAGTTTCTGCTGAGTCTGTCAGGAGAACCTCTCCATTTTCTCCACCTCCATCTTGAGAAACATGGCTTA
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TGTTGAAAATAACACAGCAATTGTTACCAATTAGTGAGTTGAACAGCTGAATGTGGTTAAGGAAAGAGTGAAGGACA
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AACCAAAGAATAATAGCAAGCCCTGGGTGGTGGTAGGGGGAGTAAGAAGAGTTGCTACAGTATATTATCTGCAATATCC
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CTGGTGCCCAATTAGAGGACCCCTCACTAGTGGTATCCAATCTCTACACTGTAGCCAGAAAAAATCCTTTGAA
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TGAGTTGATGCTAGTAACCTCTAAACCTCATTTTCTGCTGCTTCCAGGTGCCACCAGAGGGACTTCTCTATAAAAGGCCT
CTCCTCACCTTCAAGTGGGGGTTCTCTAGTTGTCTCTTCCAGGTGCCACCAGAGGGACTTCTCTATAAAAGGCCT
GTGCAACCTATGCTCTTCTCTCTCTGTTATGGACTTCTTCCAGAGAGCTGGTCCCATCTCTGATATGGGACTGTC
CACTTGTATGAGTAATAAAATCCTTCTCTGATCATACCAAAGAATTGGTGTGATGATTTTATATTAGAAGCTAAAC
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CCATTCCATTTTCTCTTAGGATAAAGTTCACAATCCTTAGGTTGGCCTACAAGTCTCTACAGTTAGGTACCCCGTC
TGCTTTCTCTCTCTCTTCACTATTAGTTATGCTCCCACTGCTGCTGCAAAACATGCAGACCTGCTGCATCTCTACCTGAA
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CCTCAGAAAGGCCTTCTCTGACACCTCAGCACAGTCTCTCTCACGAGGTGAGATGATTTTCTTCAAGGCACTGACT
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CAAGGGCTTTATATGTTTTGCTTGCCATTGTGTCTCTAGTGCCTAGGCCATTCACTGACCCACAGTAGGTGCTCTGATA
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AGAATAAGTTAGGTTCAACTATATTAACTACTCTTACTACACACACATAGCTACACTCATCAATTTGGTGGCTTAC
ACCAAAAAGGCTTATTTCTTGTGCACACTGCATTCTTGTGCTTTAAGGAGATACTCCGCCCTACCTCTTTATTACA
GACCTTGGCTGACGGAGGCTACCACTTGAATATTGTCTATTTTCAAAGTCCAGCAGAAGAGAAGTTAAGAAGGTTTC
ACTCTGGCAATTAAATATTCCAGCTAGGAAATGACATACTTTATCTTCAATATACAATCCATTGGCCATAACTAGTCTCA
TGGTCTGCCCCATTTGTAAAGGGGAAATAGGTAATCATCTCATGCAATTGAGGAGGAAAGAAGATTAGATACATGTGAC
CAGTAGAAGTCTCCTTACTGACATTTAATGTAGAGCTGTATGTGATTAAAGTTAAATTTGAATCTGAAGGGCTGACAG
AAAAATTCAAGAGGATATGATTTTAAATAATTGAACGAGGCTGAAAGATGAAATTTGGACAGATTGAATGGGTAGGCCT
TGCTAGCAGGAAAGACAAATAAACAATAATCAAGAAAGTATGATGAACGTGTGCCAGGGCAGTGAGGAGTATCCATGC
TCCTTGAAGTGGAGGATGTGTGTCAAGGAACAGAGAGAGATGATTAGAGTGGCAGACCTGATTATTAGCATATCCATGC
TTCTGTCTGAAGCCTTTTTGGCCTATAATGTAGAAATATCCTTGGAGTAAGCAGTTTCAAGCATATGGTTTATAGCATATA
GGTCATCTTGGCTGGATTGCATATTCTTCAATTTTAAATTTCAAGGCTGTATTGGTTGATATGCAGTCCAGAAAGAGCT

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GGATCTGACTTAAGAAAATAAAAACTGCTTTAATCCCTCTTTTCAGCCCATTGTCTATCCATTAGCAGGTAAACACCAG
TGTCCTCGCAAGTGTGTGAAGTGAAGTGTGCTTAAATTTTACCTTTAATTGGCACTTCTAGTGACTTAACTGAGTGAAGAG
ACCTTAGCAATGATACAAAGTGAAGGATGGTATAGAATAATAGGTTTTCTGAAAACCTGAATGCAATTGAAGGGTGCT
ATTTAATAAATCTGTCCCTAGAAGCTAATAGCACGTAGTCAATACAATTTAGCCTATTTTCTCCCATGTTACATTTGTT
AGTTGTACTGGTTTTGGAAAAGGAAAAGTCATGCTGTTACAGTTCCACTAATAGAAAACAGATAATTTGGGAGGAAATT
AGATTGGAATAAAAGCACGTTGTTAAACAAGAAATCACAATAAAGTATTGAGTAGAGAGAAACCGTTAATGGAGACAGC
TATGTCATACCTAATATGCCCCTCTTTTCATTTGATCCTTAGAGGAAGCATAAGATCGCAGCTAAGTATGGTCTCTTGAGC
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CATTTTCTTATCTGTAAATGGGGATGATAACTAGTGTGCTGCTGCTCTTTCAGGTTGTTGTGAGGATTAAAGGAGATATG
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CTTTTGCATTAAAGTAAGAGACTATTCTCTCCAGAAAACCTTTGAAGTACATGATGGAGGAACAAATAATAGCAGTTTCTC
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CATTTTGTCTTTTATTTAATATATGCCATGAGTGTCTTCTCCCTATCATGATGTCAATGTTACATTACAGATTCTAAGG
AGCAGGGGCCATATCCCTTAAACATGATTTATTTAAATAACAATATAGGATTGGATGTGACTACTCTTTTGAATGA
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TGTCTTGAGTCTCCATTTCTCAACCCCTAAGTCATGCCCAAATCCTGTTCTCTGACATTTTCTCATCTCTTACTTTT
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CAAAATTATTATCTTTTATATGAATGCTTATTAATATATATATATATATATATCTCTTTTACATCTATCTAGAAAACG
TTTTAAGGGAAGAATCATATAGCTTCAAACATTTTTGTTAATTACAATATGGAAGAAAAGAGAGTTCAATTCCTTAATC

Fig. 6.152

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TATCATGTGTGAGTTAGGGGACTTTAAGCAAGTTATTTCTTAGGAATCCTGTTTCTTGACCTATCAAATGAATGTAAG
AATAGCTACCTCACATAAGTGTGAGGACATAGATAATTTCTATAAAATCATTTAGTTAGAGTCTTGGCACATAGTAGGA
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GCAATCAAATCCACTGAATTAATCTGAGCAGGGTCAGGACTTTGAACCTGAGGAAAATCACTTGAATTTGAGGCATTAA
GTGTGCAGTACAAGGTGTTCCAGCTACAAATTTCTGAATCCTATTTCAAAGCAATGGTGCAGGAGACCAGATAGCCAC
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Fig. 6 (149)

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TTGGAATAAATGATTGACTATATCAAGTTTGCAGAGTTGGTAAGTAGCTTTTTTATTGCAAAAACCTTGCTGATTGTT
GAAAGAACCTTCAATTTCCAAATTAGGAATAATTTCTGCAAGGAAAGACTATGCTGTGTGTGTGCTGTGTGCTCTTGT
GTGTGTGTGTGTGTGTGTGTGTGTGTAACCTCTTCTGTTGTGAATCCATCAGTGGTTTTGCATTGTTTACAGGATAATGC
TCAGAATCCATAACAAGGTCAGTAACTCATCTATCATTTCTAGGCATGTCTGTTATCTGATCTCTAGCCTTTCTTCT
CCCATCGTCGCCAAGTTTAGTTCTACTTGACTTCCTTCACTTCTTCCATTGACCTAGGGAGCACCTCTAAGACTGAA

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AGGGAAGTCGTGGGCTTGGTGGAGTTGAGGTGGGGAATCTATGAACAGATAAATCAGATAGAAGCATTGTTTGGTAGAA
AGAAGAGCTCATGAGTGGCAGTAGAGATTGAGTTAAAGAGTGGGATGAAAAGAATCAAAAGGAAGTAGTACGATTG
TGACCAATTGTTAGAAAGGATGAGGGGCAGTAAGTAGTCAGTGATGACCCCGAGGTTTCTAGCTGGACCTAGAAATTGT
CCAAC TAGTCCAAAAATGAAAAATTTGCCTTTAACTAAGATGAGTTTGACAAAAGATGAAGTAAATTTGAGGGTGTAG
GTAATGAAAAACAAGTTGGCTTTTTCTCCCTTGATGGCAAATTTAGTGTGTATGGATGTATACATTTGTGTATTTGTGT
GTTTGTCAATACCCATTGATTTTTCTTAGGTTATATCAAACTACTGAAGTTGTACTAATTAAGCAACCGAAGTGTATGC
TTTCAGCATGGTACCTTTCCACACAGCACCAACATCATTATATTATTTCTATTGTAGCATAAACAAATCACACAAAT
TAGCATCTTAAAAACAATACACATTTGTTACTTTACAGTTTCCATGGGTCAGGAGACTGCACCTTGACTTAGCTGGGTTTT
CTGTTCCAGGCTCTCAAAGGCTTTAAGAAAGGTGTTGGCCAGGATTGGGGTCTCATTGAGGTTCTGGCTCCTTTTTCC
ATATCAGGTGGCTGTTGGCAGAAATCAATTTCTTAACCACTGTAAAATTCCTTGAAGCTTGTCTTCAAGGTCAGCAGG
AGAAAGAACTCTGACTTCTGACTTCTAGGCCATTTTTGGAGAGCTAATCACTTGCTTAGACCAGACCAACCCCTGAAT
AACCTTTGATTAACTTAAAGTCAACTGATTAAAGGATGCTAATTACATTTGAACAATCCTTTAACTTTGCCATATTCCAC
TGGTTAAAAGAAAATTACAGATGTTGACCATCTGGGAGGAGATAGTGAAGGGCCTGAGCTATTGGAGATCATCTTAGA
ATTCTGCCACCACAATCGTGTCTATGTTTATGCAAGGTATGTTGAGTGTGTATTTATGTGTGTAGTTAATAACTT
AAAAATGAATTACATGTATCCCAAAAACAGCGGCAACTGCACATCCCATGCCTTCTTACTTGGATTAGTTTTCTCTGA
AGTTACTCCAGGGTCTGTCTTCCATTGTTAGGTTTCTTTCTGCTGTCTGGCTTCCCTTTCTGAGCCAAAGAAAGC
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TGTGATCCCAATGAGCTTCAGCAGTTTGGAGTGAGGCCATGGCCCATGTTTGTATGCCACCAGCCTGATTTTTGTAA
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TGTTCTTTAGGGAAAACATGTTGTCTTGGGTACAAAATTCAGCAATGTTTTTTACCACATGAGACTTTATGGAAAAT
TCCAGATAACAGACCTTGCCCTAATGGTATGTGGGCAACAAATCCAGAAGTATCTATCATAGCATTCTTTCTTATAACA
AATTTTATTAGAAGCCAGGCTAGAATGTTGAAGCTGGACTCCGGGCAGGTGATTCAAAATGCCATACTATTACTTTCTCT
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GGAGTTTTTATGCATACATGTTTCTCTACACTGAATAAATACCTCCAAACCTACCGTCACTGCTTATTAACCCCTACT
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GATCGTCAGCTCCATGAAGGCAGGTTGCAGGCTTGTTTTTCTGACCCTCTATCATCAACCCAGAGCACATAAATGAA
CATAAAGTGAATTCATAAAAATGTGCTGAAAGGATAAACTACTGTTATTTACTACATTAATTACAACCTTTGTTAGCC
CCTTACAAAAGATGCTTTCTTAGATGCTGCATTTCCCATTTACAGAGGTTGAATAATATGACTATGATAGTTTTAGAG
GAGATGGGAAATAGTTTGGTATTTCTTAAATTAATAATGATCTGTAGAGCTAGAATTTTATTTCTGAAAGATAAT
CTTGTTTAGCCAGTGTGAGAAACTACATTACACAAAAGAACATTTTGTCTAGTATTATTTTCGAGGAGAACTGTTGTT
AACTGTGCTTCTTAGAAGATTGTTTTATCAGGCACCGTTGCTTCTTTGAGCTTTATGAACCTCATGTTTTAGGACAG
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CAATTGATTAATTGGGTTGACTCGATGATACAAGCACACGATCTTCAGTATCAAAGATACTCATGACCAGTAAACCCC
TCTTTAGTTCTCAATTATCCAATTCCTTTCTGGAGGCACTGCTACTCTTGTATTTTCTAATGGAGATCTTTTATGT
GTTTACTCAAATGCTAAGACATAATATATCATATTCTGCTTCTGCTTATCTTCCCTAATGATATAGCAAGGAGATTGA
TTCCTAACTCTGATGCAAAATCTGCCATAGTGCTTTTAGCAATTTGCAAGTATCGTCCATGATCTGGATGAGACATAATTT
CACCAGACTCTGTGAGTCTGTTGGATTGTTTCCATTTGTTGCTCACTACCTACAGTGTAGTGACTTACTTTT
ACTTATTTGTTTCATGTAAAGTAAATATATCTCTAGAATTAATTTTGGAGGTGGAATTTCCCAAGTCAAAGGAAGGAAAT
ACATATTTTTTAAAGGATTTTAGTAGATTGTTCTGAAAAAATTAACACCAAACTTACCCTCACATGACTATTTTTGCT
TTTTCTCAGTATACTTTTAAATAGATTGCATTATAAAATTTAATATTTACCATTAAATAAGTGAAGCAATGTCTAA
TTGCAAGTTTTAATTTTATTTTATTAAGGAAGTTAATCTTTTTTTTTTAGCTGAAGTCGAAAAATATGTTTCATCTCTTT

Fig. 6. [6]

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TTCATTGAAGCTCATTAGTTTTCCAAAAATGTGTCCTCATTTCATACAAAGTATCTTTGAACCATTGCCAGGTTCATAGAA
GTGACTCTTGAAGATCTTCTTCTCTGGGATCAAGGGTCACATCTCGTTCTCTGGCTTCATACCCCTTCTCTCTACAC
ATCTCAATTAAGTAAGCTTCCTTTCTTATCCTCTGAAATTATAATCATCTTTAGGCTCCCAGATATAGGTGACCTGTGC
CATCTAGTCTGACTTCCAGTTTGCAAATATCTTCAAAAAGTGGAAACAGCTTTTACTTTCTACTTTATAAATTCATAC
GTGCTCAGGAGGACTTTTATTAGATTGGGACATATTGCTTAGTTAGTCTCTAGGGTGGAAATCATAATTAATGAAAGAA
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GAAGAGACACTTGATTAGGCAGATCGTGACAACTTCAGCTTTGCCCATCATACATCCTCCAGAGACTCCTCAAACCTCAA
GACAGACTTTCTACAGGAAGGAATGGCTGTTTCTATTTCAGAATATTACTGATACCAGAGCTTTGCTGCATCTTCATGT
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CATAGTTCACTGTCACCTCAAACCTCTGGGCTCAAGTGATCCTCTGCCCGGCTTCCGGAGTGCTAGGATTACAGGA
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CAATTCCTTCTTAACTCATTTCCTGAGACATTTTGGAAATTTGCAATATATATGAAAAAGCACTTATTCTCAGTGATG
GAAACACCATTTATTTCTTTTACTAGAATGTGATCTTCTGGTTTTAATATTTATGTCTCTGTGCCAAGGTGAAAAATATTG
TAGAAATGAAAGTATAATGAGAGAGAATCCAGATAATAAGATTTTCAATAAACAAAAAAGAGTATGTATAGGAGTCC
TGTCTGCTGCAAAATTTAAGAATGGTTTTCTGTCAACATCAGTTGTCACTTGCTTACTGAAAACCTCTATAAAAAACAA
GACACAAATGATTGAGAAGATAGTAGAGACTGAACTAGAACTGTTGATTTAATAAGCTTTCTTACTCATATACTTAAT
CCACAGTAAATTTATAGCAAATTACATTTTCATAGTATTTCAGAATTAAGATCATAGTCAGGGCTTAAAGGAATCCA
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GCGTACAAAGACTGACAAATTTAGATACAAATATACTACTCTGTCTCAAAATAGTGCTCTTCTGAAGCTTTAGTCAGAA
ATTATGGTGACATTTCTGCTAGATTTGTCTAGTTGCTGGGTTTGAGGAAGAACCTAGGCTACACATTTCTCTTGGC
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ACATCGCACTAATGCCCATGACTGCACTGCTTGAACCATGAGGTTATTGAACAGAAAGCAAATCCTTTTCTGAAGAGCC
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GACTATAGAAAAATCATTTTCTTCTTAAATGTTGTCTTAGTCTTAAATTAGAAAAAATAGTAAGAAATTAACAAAA
CAAAAAGTTAATAGTATTATTACATATATAAATAATGGGCCATCATGGTATCTGATTTTGATAAAAGGAAAAATATACT
CTGGGATTTATTTTCAAATCTGGCACTCTATTTATGAGTACCTAGACAGACACTAGAGTCTCATGGAATTTAGTATT
AGAAATGCTACTTTGGAGTACATTTGATGTCAATCAAGTTAAAAAATTAAGGAAGTAGATTTTCAAATAGGTAGCATA
AATGTAAAAATTTGAATGTTGGAGCAAGAAGATCATCTAATGCCCTAGCAGTTCTTAATGTTGCTTGGGTCATGGAT
CCCTTAAGAACACGATAAGAGTTTACCTTCTTCTCCCTAGAAAATATACTGCTTTTCTTTTATAATTAACAATAA
TATGAAAGTTAATCTTAGTCCAGAGTCCTAATAGCCCAAGAATAGAAATGTCAAATTCATCTCATATCATTAGTTTGT
AATTTCCGTTGCCCTTTTAAATCCAAATAACACAGAAGCTGTAAGAACTGCTTGTCTCATCTGACTTTTTTATTTGTCTTAC
CCAATTGAGCTTCTTATATTACAGATAAGGAAATTGAGGCACAGAGATATTAAGAACTTTTCCGCAGGAAAAAATC
TGCTAGTTATTGAGCTGGGATTCAAATTCAGGTAGCTGATTCTAGTGTGCATGTGCTAACACAGCACATACTGTGGCT
AACCATGCTACACTCATGCTCAAACTACGCTGTGATAGGTCTTATTAGCATCCCCATTTTACAGACGTGGAGATTGA

Fig. 6.162

Fig. 6.163

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CTCCCTTATATTTCTTCAATTCTTATCATTTGAGTATCTAGTTAATGAGTTCTCATTTGATGTTATAGATTTTTTTAGC
TCTTCCTCACCTGTATTCCTTGGAGTAAATTTTATGAATCTTAGAAACAGAGTCTGGCTGATAAATTTATTGGTGCATC
AAACTCATTGAGAAATGTCTTCCAGTTTCATCTTCCAAAATGTTACTAGTTCTAAAGGTAGTGGTTGATAGTTTCACACA
AAAAATCAATACTGATTGAGAAATAACTTGCTCATCAGGGAAATAAACCTGGAAGGTTTTAGTCATAATTTATTTATA
ATCCCTCCCTATGTGATATTGAGAGATGAAATTTGAGGTGAAAAAATTCAGTATTCTTTTTCATATATTTTGAAAATA
TAAGAGTCTTTGATGTATTTCTGGGAGCTAGCATCTTGAAAAGAGGAATAAAAAATGACTTATCTGGAGTTAGGAAGGTG
CTAGTCCATCACCCATAAACAGTTAAAGAGATAAAGGTGATTTATCAGAAAGTACAACAAAGTGATAACCAATAGAAAT
ATTATTTCTCCATACCAAGCAAGTTACAGGTATCCCTGCTCTGGACAAATGCGTGATGTGTAAACCCAGATTGACCAAG
GGCATAAGGGAGATGTAGATCTGTAGCTTTAGCACACATGACCAAGGTGGTGCACCTTGGTGTAGGCTCTCGGCTGATTG
CTGACTTTGACACCTTGCGGGCATTTTCATGAATCTAAATTCGGGAATAAGATTTAGAAAGCTGGCAGATGACGAAGAGA
TTGTTTTGGGCAACCAGGACTCTTGATGTGACATAATCAATGCTGAAGTTAGTTTCATCATCCCATTCTTGTATTTCCA
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TTCTTTGAAATATAAGCTTTTGAAGAATATTTTAAAAAGAGTAAACATTTTAGCTTTTCATGTAAAGTTTGAAAAA
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CATTATGCAAAATTAATGCAAAATTCAGTGATGAGAGGAAGAGGTCTCAGAGCATTGAGCAGATGCCGTGTGCGTAATA
GAGTAAGGCATTACAAAATTACTTGCAGCCACTGTGACTGTGTATTTTCCCGTATTCTGAAAAGAAATCTGTCTATGTG
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GAAATAGTGGTATTGAAGTAGAATTATGAGCTCTACATTGAGTCTACTTTTACATCGGATTGTCTCTCATTTTGGGA
GGGTAATTTCTTTAGATTCTCTAGTCCTAAGCTGTAATATGTCTTATAGTTGTCTATTTTAGTGAACAAACAGAAA
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AAATGCTCTTCTTTTTCAGGCTTGCCCTTCAAAAAAGTTAGTCTCATACTGAATGGCAATAATTTCTTCTCTGCTC
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TTTTTCTGTGGATATAATCCTCTGGTCTTAAAAATTAATTTTAGTAATGGATTTTACAAGTTCCCTTGGAGAGCATG
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TCTTAAATATTTGTTATTACGTGTATCTTTGTAATAAAAGTGAGGCTTAAGAAGTTTGACTTTGTTTTAGGATGGAAC
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TGGCAACATTACCAGGAACTCATATACACACTCAAATTTGCCAGGCTCTGAACCTCTATAATCTCCAGCATTCTGATTTT
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CAAATGTACCATAATGGGAATATTCTATAAATATGTAAATTTATGTCCATATGATAGAATACTAGGCACCCATTAAATC
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CTAGTTAAATAGACGAAAATATAAAATTTTAAATGCGAGAAATTTCAATTTATAACATATGCATAGGAAATGAAAA
GAGTAGTTAGCTCTAAATATGGGATTTATGGATAAATTTATATTTTCACTTTTATTTTCAAAATTTTATAATTAG
CTTTAGGATTTTTATAACCAGAACAAATTACATTTAAGACTCCCTTCTAAACTTATTTGCTTATTTTCAATCTCAAAT
GTGAATTTGTATGGCCTCTATATTTCCAATCTAATTTTACAGACATTAATTTCTCTGAAATTCAGTGAAAAATTAGC
AGAGTCGAAGTTACACTTCTGTATGGCATTTAAATTCCTCCTCCAGAATACAGCCACTGTTCTACAGTACAGAGGAGT
CCTTCATATCACGATTTTTCATTGTGTCTAAGAGGCATGTGTTTCCACTTTGTCTATTATTTGGTCTAAAGGATTTTTCT

Fig. 6.164

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TCTATTTTGTCTGTGTTTTTGTACAAGACATATAAAGAAAGGTGTTAAGGGAGTGTTAAATATAATCAAGGTGTTAAGG
AGAAGAGCCCAGAGAGAGAAGTCTTAATCTTTTCATCTGGTCCAGTTGTCAAAGTCTTTTGTCTTTAGCCATCACTTTT
TTAATCAGAGACATATAATCTATTAACTATGGGAACAAGAACCACAGATTTAGCCTGTGCTTATCTGGAAACCTCCCCCT
AAAATACTACTGAACGACTCTTTTTTTTTTTTATTCCGTGTCTTTTTTCTGTTGCAAACCTGATTGTCCACTATAACCTGG
AGAGGGGACTCAATTTTTTAGAACTGTCTTATACACCTCTTAATTAGTTACCTATTGCTGCATATACCCAAAATTTAGC
AGCTTAAACAATAATGAAATTTTATTATGCCCACAGTTTCTTTAGGTCTGTAGTAATTTAAGAGAACTTAGCTGGGT
GATTATAGTGTACTCTCTCATGATATTACAGTCATATACATCAGGACTGCATTCTTCTGAATACTTAGACTAGAGCTGGA
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GGGTGGTCTTCTCCATGGGGCAGCTTAAGGATCTTCATGGTTTGTGGGCTGTTTCAAAAGTGTGCGTGATCCATAGGA
GATTAAAGGTGGAAGCTGCAATGTCTTTATGATAGAGCCTCAAAGTCAACAATGTCAATCCCAACGTATCCTTTTTTT
TCACATAGGTGAGCCCTACGCAATGTGAGAGTGAGGAAGGGACTACAGAAAAGAATGAATTTCCAGAGATGAGTAGGAT
CATATTGGGTGCCATCTTGGAGGCTGGCTACCAGTGAAGAAGACTGCTTTCTGGCCCTCAGTGATTTACTTCCATCTCA
CATGCAAAACATACTCCCTTTATTGTGGTTCCCAAGAGTCTCATTCTATTATGGCATCAGCTCGAAGTCCAAGATCTTA
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GGAAATTACACAAGGCCCTGAACATGAGGAGATGGGATCTTAGAATCTGTCTAACATACCAGGTCTATGTGCAAAATAAG
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TTCTCAGTTTCTATGGGTCAAGAATTCAGAAGCATCTTTCTTCAGTGGTTATGGCTTTGGCACTCATATGAAGTTGCAG
TTAAGAAGTCAGTCAGGGTGATAGAAATATAAAGGCTTGACTGGGGCTGGAGGATGTGCTTCTAAGGTGATTTACTCAC
ATGATCAAGTTGATATTGGCTGTTGCAGGCAGGTCTCATTCTTCCGCAAAATGAATGCTCTCCAGGCTGCATGAGTGT
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Fig. 6. 167

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Fig. 6. 169

[illegible]

Fig. 6.170

[illegible]

Fig. 6.177

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Fig. 6.175

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CCAACCATCTCACTGCAGAGAGCTGGCACTCAACAACTCATAATTTTTCTCAATACAATTCTCATGACATAACCTGA
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Fig. 6.180

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Fig. 6: [8]

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[illegible]

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ACTCACAAATTAGGTTTCTATTCTGGGGCTATTTTGTACATTTGTGTACAAAAGACTGCATTTTTAAGGTGTCTGACTTT
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Fig. 6.19f

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AGCACGGAGGTATGGGCTGAGCAGCGCTGCCTGAGGAGGTGATGTACAAGTGGGTATTTATCCCCCGGGCAAACCAG
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GTCAGTGTCTCTTGTCTTTAATGATGGTCTTGAACACCACCTAGTTGTTGTGACTTAAAAACAGAGTTGTAACCTGTTTA
CAGGTTTATATCCATTAAACCACTTGATCTTGTGTTTCTATGCTCTCTTATTCTCACCTTAGCATTTAGTTACCACCTCAC
TAAATCAGTTGATCTTGAACCTTTAGAAATCTTACCCTTACCTGAGGGTCTTGTTTACAAAGCAGATTTCTAGATGC

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TCTTGCTCAGGTA CTCTTATTCTGTAGTTGAAATGGGCATTTTAGGGCCAGACACTTTGGCTCATACCTATAATACCAA
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Fig. 6. 193

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AGGTTACCATCCGTTTCCATCCTGGTCATAGGAGGTCACTCCTTCTCCCATAGGTCCTTGCCACATGGCCCTAGCCCCACA
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Fig. 6. 192

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Fig. 6.200

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CGCCTCGGCTCACTGCAACCTCCACCTCCCGGGTTCCGCCATTCTCCTGCTCAGTTTCCCGAGTAGCTGGGACTACAGG
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Fig. 6.201

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[illegible]

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Fig. 6.210

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Fig. 6.214

GGTGTAAATTTGCATTAAAAATCAGATGAGATTATTTCTTGGTTTTACTACTAAGACACATGGACACAGGAAGGGGAACAT
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GAACTGTATTGTTGAAGAGCTTTTCTTCCTCACAATTGAAGAAGACATAAAGACTGGTGATATAAGAGCAAATGCAGAC
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GGATGAATCCCACTTGATCATGGTGAACAGTCTTTTTATTGTGTTTTTAAATTCAGTTGGCTAGTTTGTGAGGATTTT

Fig. 6.24

Fig. 6.218

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GTTGAATCATGAAAAGTCCCAACAAAGCATTGCTGGTTTTAGGTGATGAACTAGGTGTTAATGAATTTATTTTTTC
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AGAACAACCCAGAAATTTAAAATATAAATACCAGAGTCCAGCTTAATCTGAAATTCCTGTTGGGGCCAAATACAAT
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GGCTCAATGTTAAAGTCAGCCATTGTTTAAAGGCAGAAATTCAGGTTTAGATATAGTGTAGCAAAGATTTTCCATTATAT
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CAACTAGGATTTATGTTGTGTAATAATTTATACATATATCTCTCCATATATGATTTCAAGGATAGATATATAGAGTC
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GCCCTGGGAAGGCTGAGAACTGCGGGTATACTCCATAACAGATCCCTCCCCAGTACTGACAGTGGTTCCGGTGGGTGAG
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TTTTGTTACAATGAATGAACCAGTATTGGTACATTATTATTATTTAAAGTCCCTATTTCATTATATATCCTTAGTTTTA
CCTAAGGTTCTTTTTTCTATTCCAGGGCCCCACTCAGGATACCACATTGCATTTAGTTCTCGTGTTCATTTATCTTT

Fig. 6.224

Fig. 6.225

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TAAAGCCTGTAGAGTACATGCTCCCTTCCTCCACATCACACTGCCCTTGCTTGTTCACACTCAATGTGAGAATCCACTT
CTCAAGTTTGTCTTGTCTTATTCCAACCTGTTTTGCAATGAATCTCTTCAACAGCTCTATAGTTCTGGCACTCACTTG
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GCAGATAATTGCATTCTTATTGAGAGTTATGTTAACTAGTGTGGGCTTTTATTTGCTTCTCTCTTCTATCAATGAGT
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GGTCACAGCTAAGCACTTTGTGTGGTAACCTTCTCTTCTGTCTCAGGCCTGCTGAGATCATCTGCTCAGCAGCATGTC
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CAAATGTGGATTGAGAACCCGACAGGATGCCACCATGCTGGCTAATTTTTTTTGAATTTTGTAGTAGAGACGGTTTTTGCCA
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GGGTCCCAAATTACTGGAATCATAACATGGCTCATGTGAAGAAAGATTACTATTTTCAAGCTGTTGTATGTTAA

Fig. 6. 226

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CTATTATAGTAAGTAGCATAATTGGCATATATTTTAGGCCGTATTTTCAATAAATCAATATTTTGGCTCAAAGGCCAAA
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Fig. 6.228

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[illegible]

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GCCAGTCCAGATGCTCTCCCTTTCATGCTCCCTTATGTTGTCATTAATGGGTGACTGCAGAGAACCATAGGCATTTGAGGAC
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GTGTACTTTTTCTAAGCTCTGTGGTCTGCCTTCTTAGGACTGCTTGCCTGTACTGTTATTTCAAGAAATGTTTATCTAGT

Fig. 6:233

GAGGAGTGTCTGCATTGCTCTCATTTGTGTGCATCACAGGGCCACAGGAAGCAATGGTATGACATTTTTGCTGCACATCTATTTT
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CCAATTTAGAGGATTTGCATTTTAGGACTAATTAATTAAGCTAATTGAGCAGGGACTGAGTTAAGCCTACTGATAGT
GCTTCATAAATATTTCATATTAACAATAACAAGATGTTCTTTTCAAGGCTAAAACTTTTTCTAAATGGTGTATACA
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Fig. 6.234

[illegible]

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Fig. 6.239

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Fig. 6.240

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Fig. 6. 243

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AAATAATTTCTTTAATTTTGTTCATAGATTTTAAATTGAAATTGGTGAACATATGCACACATACACAAAACTGTAGATTT
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Fig. 6.248

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Fig. 6.2'54

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TGTGGGAGTGCTGGTGAGGGTCCTTCCCTTGCTGCCTGCTCTGTGCTCCCTCTTTCTGGTGATGCAGCTGTTCTGGTAT
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Fig. 6.256

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TAAATAATACCTAGAAATAAAAAGGAGTAAAGTAGAATAGTTTATCTGTTGTACTAAGACTTCATACACAATATTTCTGAAAAGTGGAATATCTCTTGGGTGAAATACTTCATATATGTATTGTATGTACATACATGCAATATGTACACACAGAAATATATAAAATATATGTATATGTATACATCTATATGTACAGAACATATAATTTTATTAGCATTTCTTGTAATGGCATTTTATTATAAATCCAATGACCTCAATTATTTCTATGGGTAAAGTGCCTAAGTATGTCAAGAGAGCTGGCATAGAAAAATGGAAAAAGCACTACACCCCTTAGCTTGCAAGTAGATGTGAATTTTCTGGCCTTGACTTTGTTGAATCTGTGTATGAATCATATGTTTACTCTGATTAACATAAAAACATCTGGATGTACTAATCTTGGGGACACATGCTTCATATGCATGAATGCCTGAAAAATTGTGAGAAATTTAGATTTCTTTTCTTTTATAAATGACTACTACCCGAATTCCTGCAATACCTAGAGAGTTACAAGTGCTTAGCTCTGACCTTTTATCCATTCAATTGAAGTTGTCCACCTTTAGTTTATTACACATATGACTCTTAGTAGAGCAAACATCTGGATTATTGTCAACAGTTCTCAAACACACCATTGGATTTCACATACAGACTACGAACGAACCCCATGGAAAAAATTCAGGCATACAGGCCTACACCAGATCCTGAATAGCCCCTGGTTTTCTGGTTACTATTTTCTCAGGCCAGATCCAAGAAGTCTCTTTGGGCTTGCTCTCTGGGATTCTCTGTATAAAATTGGCTTTAGATTGAGACTGACGTGAAGATAGAGCTGGTCATGAAAGACAGAAACAGATGTGAATGAAATAATCTCCTTTGAGACATAAAAAATGTAAAGATATACCAAGAAAGGGGAAATGAGTTGTTTCATTTTCTGACAACCTGTGAATTTGTTGACCCGGACAAGAGAGTAGGGAGATTACAATGTATCATGTTTCAGGGCCCATCTGTGCCATGAATTAAGACAATGTTGGCATTCTCCTCAGTCATCTTCCGAAAAATAGAGATTTAGAGTTAGGAAAGGAATAGCCTATCCCATTAACCTACGCTCACTCATCTCATGCAGGTGACCTCTCATTTATGTATTTCTCAGGATATAGACCAACGCTGGAGTATAGAAATATTATGAGACACTTCGTAATTTTAGGTCTTCTGGTACCACATTTAAAAGGTAAAAAGAAATAGGTAAAAATTTATTTCAAGGATATATTTTATTTAACTCACTGTATCAGATTTATTTTAACTTGTAAACAATATATATAAATTATTAATGAGATATTTAAACAATCTTTCTCCTACATCTTTGTCTTTGAAATCCTGTATGTATTTTACTTTACATCACATATAATTTGGATGCCCAATTGCTCAATTATACCTGGATCTTTATTTGGACATTTAGATCTCATAGATTTTACAATAGAAATATAGATTTCACTTTCCCAAGTTGTTTCCAAATATAGTATTTAATAATCAACTATCAGTCTTTTAAATCATAAAAATTAAGTAAGACTAAAAATTTAGTCTTTTAGTCATACTAACCCTTTTCAAGTGCCCAACAGCCTCTTGTTGGCCATTGGCTGCGGTATTAGACAGTTCATATTTAGATTATCTGGGTTTGAACCTCAGAGTCTGCCACTCACTGACTGTATTGGCAAGTTGTTGCCAACCTGGGCAAGTTTTTTCTCTTTTATGCTTCAGTTTCCTCACCTGAGCACACCTTTTTCTTGAAGAAGCTCTCCTGAAGAAAATCCCGTATTTTTCTTCAGGACATTGCTATTTTCCACTTAAACTCAGAACCCTTAGAGTGATCACTGAATCCTCTCTCCCTCACTCAGCTTTTATCTGCCACAAGGTCAGTCTCTGAGATGTACACTGTAAAACTCATGCTTCTCTGTGACTTCATTTCCACTGCCACTACCTCTCCTTTGCTATGATAGCTGCAATGATCTCCTATTTAATTAGCCTCAGTCCAGACTCTAGCCATCACTCCACATTATGCCCCCTGGATGCTGCTACTAGTTCATTTTCTGAAATGGGTATCTTTATCATACTCTCCACAGTTCAAACATATTTCTTCACTGCCCGTCACTTCCAGCATAAATCCATGTCTCTGTGAAACCTTTTGTGACAACTTCCATGCCAGATCCAAATGACAACTCCCTTCTTGTGCTGTCTATGGCCCTCCACACAGACTTCTGCCATAGCCCCAGAGTCTTAGTCCACATATAGGAGTACATGGGCTCTAAGCCCTACACAGACTATTTTGTGTGTGTATTTTTGTGTTTTGTTTTGTTTTGTTGTTTGTTCGATATCTTGATATCTCTGTAACAGAGCACAGTGGTTTCTTGACAGTTTCTCATTTTTCTGTATGAGCTTAGTGAATGACATTCCTTTGTCTGGCATTCCAGGCCCTCAGAATTAGCTCCACTTGACTCTTCTGGCTCCTTCTCTGTCTTTTATCCACACAGATCTCTGTATAGACAACTGGTGATTACTGTCTCACAATAGTGTCCTCCCAATGTTTGTGTCTCCATGCCTTTGGCTCGCATCCTCTCCTCTTCTGAAAGTGTCCCTGCATTTCTTTGATCTGCCAAATTTCTACCCTTTCTTCAAGGTCCAGTCCCTCCCTCTTCCATGATGAAGACTGCGGAGCATGATGCAATTCAGTGTGTTTCAAATTCAGGATTGGGTGGCCTGATGGAAGGAGGCCAGGCTTGGGGTCCAAGTGGATCTGTTTCTTCTGTTTTCCGCCAAGTTGCCTACCTCTCTGAGGTTACATTCATATCTTTGAAATCACAGTAACAAAACATAAGGTGCAGAACTATCTTGAGTATTAAAGAATAACGTGTGCACTGGCATTCACTGAGGGTTCCAGATGGAGCAGCTGAACCTCTGTTACTTTTCACTACAGTCATAGGATATGTAATCATGTAGTCTCTAAATGTCTTTTCACTTGAAGATTTTGTGCTCACTTTGGATTGCTATACAAATCATGTCTGATTATCTAACTAGACTGCAAGTGTCTGGACTGAAACAAATGCATAATAGAATTCGAGCAGATATGCACAGATATATATATGTGTGTGTGTGTATGTGTATATTTGTGTGTGTGCACATGTATTTGTAGATATCAAGGGAGAGAGGCACCTTTCATGTGTATGTATTATTAGTCCCCACAAGAAAGCATCATTTCTAGGAGGAAGTTGAAATGTCCCTATTTTATCTATGAAGACTCTAGACTTGAAGAGTTTGTAGTCTTCCCAAATCAGTCCAACCTTTAAAGTGATAGAAGCAAAAAATTCACAGTGTTTACACATCTTTTCCAAAGCTGTTTGATGTTATTTATTTCCCGCATATCACATCTGTGAGATTATCTGCCTAGATCAATATATGTGCTAACCATCTGTCAATCTTTATCTACATGCAATTTGTACCAGGTTCAGGGATTGTGGGAGGGAAGGTTAGGTGGTTGTAAAGAGACATGACTAGTATGGTAGGCAAAATACAGCACTGCGACAAGAACCACTAGGCATTTCTTTTTTTTTTTTTTTTTTTTGTAGATGGAGTCTCACTCAGTCAGCCAGGCTGGAGTGTAGTTGGCGCGATCTCGGCTCAAGCTCTGCCTCCCGGTTTACGCCATTCTCCTGCCTCAGCTTCTTGAGTAGCTGGGACTACAGGCCCCACCACCTACGCCCGCTAATTTTTTTTTGTATTTTTTAGTAGAGACGGGGTTTACCCTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCACCTTGGCCTCCCAAAGTGTGGGATTACAGGCGTGAGCCACCGCGCCAGCCAACCATGGCATTTTTTGTGTAGGTCACACTCAGCATTTGGTATCAAGAAATAACAAATGACACTTTGAGTTTCTTTTTTCTGAAAAAGGGCAGGAAGAGCTTAATAGCAAGTGCAATTTGCCACAGGCAACAGTGTATAACTGGAAAACTTAAATGTAGATAATTTTCTCAAATGCTTTCTAACAAAAGATAGACAAGTTTAAATTTGGCTGATTTCATATACCATAATATAGTACCAGCAATGCCAAGATACAGAAAATCGCTTAAGAAAAACAGAGTTCAGAAGACTTCGGATAAAATATCATCTTAAAGCTTAACCTTATACCTATAAAAAAATTCAGGGATATCAGTTATGACTTCTCATTGAGTAGTCTCATGTTAGACAAAATAGTTTCCCATATTTTGGTGAAGGACCAGATAAATTTACCAGTAAATGAAACAATCATTTTCTTTTTTGTCTTATACTCATTCTGCATGTGATTTGTATAGGGGATCAAGTCAAGAGATTGCCAGTACAAAGTAACAACCTCTCATTTGTATTGCTTAAAGTTAATCATTAATATTTTCCATGGATCAATACCTGTAGAAGCATGAGATGCAGCAGTGTATCTTCAATTTTCTATGTGCTGCCAAGTACAGCAACGATGGGCCAGATTTGTGGGACCGTGCCATGAGCTACAGACCTCAGCTCCCTCTATAACTGATCTTCCCCCTCAGTGTCTCCCACTGTCCTTAAGCAGAGCGTAATTTGTGGATGTGTAACTACCGCAGAGGGGAGTAGTCTTTTATGTTGTTCAATTTCTTCACTTTTCACTTTT

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Fig. 6.262

[illegible]

Fig. 6.264.

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Fig. 6.265

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CATGATGTAATGCCCTTCTTTGTCTTTTTATCTTTGTTGGTTTAAAGTCTTTTTGTCTAGAACTAGGATTGCAACCC
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Fig. 6.274

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CAAAGGAGATAGGTAATGTCTTCTCTCAAGAGCCACTAGACCACAAAATATCAACATCAAAGATGAATGCAAGCGCTA
GGGGAACGATACAGATCAGAGGCACCATGTGTTGTGCATTCTGGTATTTGTGAGAGATAAGGAATGAAGTGAATTTT
TTCATGGATTTGTCTTGAAGGAAAAATAAATGAGAGTTATGCTAAGTATAAACTATCTTTACAAGTTCTCTTCCGT
TAAGACCTTCAAAGATATGTAAATCATATTTGAGAATTGCTTTTAAAGGACCTAATTCAGGTTAGCTAACAAATCATT
ATCTAAAAAGAGAGTGTAGAACTATGCAATTTAAAGTGAGTAGTTCACTTCATGATTGAGCAGAAATTCCTGAGTTC
AAATCATGGCTCTTCCACTTGACGCTTATGACATTTATGAAATTCCTTAAGTGTGCTCTGTTTCAAGTGCTCTGCG
CCTACATCATTAGAGTTATTCAAGTATTAAATGAGATAATACATATATAGCCGTTAAACTAGCGCCTGGCATAGAGTAA
ACAGTCAGCTCACATCAGCTAGCGTTATTCAAGAATAGGCTATACATTTCTTTCCATCAAAGATAAGTGCAAGTTTG
AATAGAAAAATTAATTTATGAAACAAGACTGATTTTATGCTTTTCTATAGTGCCCATGATTCAAAGGAAAAAGAGAAA
TAATCACCAACCAAGTGACCTGTAAAAATAGATAGGAGTACGCCATGCATACATACATGATATATGATTTTGGGA
TATATATGGATATATTTATGTGTGTTTGCATACGCTATATATACATACATACCTTTGCCAAGAAAAATGAGTGTCT
CATATCTAAAAAATTTTCAAGTGTAAAGACTAGCCAAGTGTCAAGTACACAATTTGTATTCAACATATATTTACAGAAT
TTAACTTTGTAAAAATTTCAAATCACTGTATTGCTTTTCTACTTGTAAAAACAATTACAAAAATCCCTTGGCTTTTGT
GGTGTGGACTATTATAAGGGACTCTGATGCTTCATGACAGGGAGTAATTTGATCCAAAGTACAACGGAGCTSTCGTGTG
GATTTAAGTTACCAAATATTGAAGGGACCCATGCACCACCAAGTATTCAAATCACAATATAATTTCAATATTCTCTAC

Fig. 6.272

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CTCCCTTTGCAAAATGTAATAACTCTGAACCTGATAAAACACATTGTACCTGTGTGAGAAAAATGCCACCAGGAAGGAGCTG
ACTGCTAATCTAGGCCTGTGCTGTCCAACAAGAGAGCTAGTAGCCATAGAGCACTTGAGCTGTGGCTAATCCAAATCAG
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GGAGACCCAAAGTCATCATCTGCTACTAAGGCTGGGTCTTGGAGATGGAGAAGGCCGTAGAAGAAAGGAAGCCAAAA
AAGAAAGGAATGAGATTTTGATATTAATTAAGTCTATACATAAGCCCTGGACTTTTGTGATGCTAGGCTAAT
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GTGAGTAAGCAGTTTGTCTAAGGGTTGCATAGTTTAAAAAGTAGTCAATAAGCTAAAGTTTGAACCTGAGATGCCTGGC

Fig. 6.273:

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[illegible]

Fig. 6.274

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GAGTGACTGTGTTTTATTAGAATTGCAAGAAAGGGAACTTGAACATAAAATTTAACATGGGGCAACAGGCCCTTGATT
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 AAAGAAATGTCATTCTGGACTGAGAGCACATACCATAGGGAAAACATGAGGCTTATGGGCTGAATTGTGTACACCCAA
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 CCTCTAGAATATACAGAGCTTTATCTCATTTTTTCCAGACGAAGAAATGGACCAAGAAAGACTAAACAATTTGTTCAACT
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 GTTTCATCTCTGTTTCTTCCCTTCCCTTCCCTTCTGGCTCTGTTGCTTAATTATTGACCCAGGAAGCCTCAGAAGGGGCCA
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 CTACCAGATCCAGAGACCATTAGTTATATGGGCACCTGAGTTTCTGCTAAAGAATCACAACCTCAATTTAATTTTTTTT
 TTTTTTGGTATTTTCAATTCAGCCAGTCTGGAGT
 TCTGTATTAACATATGAGACAGCAGAATAAATGTGTCCATTCAATTCATAGATATTTCTCAAAGAGTTGCCAAGAAT

Fig. 6.275

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AAAGAATAGTGCTAAAAAAGGTGGAAGATGGTTAGGTGTAATAATTACCTCCTCTCACAGGACCTGGCACATGACAAAT
GCTTCAGAAATATTTATCAAACCTGAAATGAGCTATTACATGAATTGTATTCTCTGGCTTTTGAATCTATTGGGGAAATG
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TCAATTTGGACTAGCCATATTTTTAAGTATTTAATAGTTATATGTGTCTTGGGGGGGGCTACTCTATAGACTATATGGCT
CCAGAATCACAGAGAGGGGAAAAATAATAATGTATTTTTAAAGCTGCAGTATTTTTTACCATGGGTATATTTTTAAGTTT
GATTGTAACACAAAAGTACAAAAATGAAACAACTGACTCAACACATTTTTTTTTCTTTTTTTTATTATTACTTTAAG
TTTTAGGGTACATGTGCACAACGTGCAGGTTTGTACATATGTATACATGTGCCATATTGGTGTGCTGCACCCATTAAAC
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Fig. 6.283

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Fig. 6.284.

[illegible]

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Fig. 6.29

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CAGAAGCTTTTAAGTTTATTTAGATCCCACTTGTCAATTTTTGCTTTTATTGTGATTGCTTTTGGTGTCTTTGTCATGA
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Fig. 6.292

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CAGTTGAGAGCGTGGATGAAAGGTAGATTGGAAGTGTATTACCATCCAGTAATCCACTGAAATGGAACAGTGTGGAGA
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TGGGTCTGTGGAGTAAATGGTCATGAGTAGTGCTAGCCACACACATTTCTTTAACACTTCTGAATGCAGCAGGCCAGCTG
GTCATTACTGTTACTGCCCCCTCATGGGAGACCAAATATAGCTATATCATTCTCTCCCAAACAGCTGCTTCTGGCAAAA
GTTATCTCTTTTAAAGAAATGTGTGCATTCTTAAATGTCATTAAAGGAAGCAAAACATATGGAACAAGAAAGCACCTCACT
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ACCCGCCAAATGGGTTACAGCAAGACTTTTTTACACCTACTGTTATGTTTCAAGTATGGAAGGCTGGGGATTGCGC
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AGAAATCTACTTTGCCAAACCCCACTATTTAGAAGTTTTAATGTCTCCAGTTGTGTACTAAATACCTTCAAAACC

Fig. 6.298

[illegible]

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TTTCAAAAAATCATAAAAATGAAATCCTGATGTTTAGACATTTTAAATGGTAATGTTTTTAAATGCCACAGTATAAAAA

Fig. 6. ~~3ec~~

TAAAGCATTGATTTAAATGACACTGCTACAGTGTAGATGGTGCCAGCACCCTTAAGTATCATTGCCAGTGTCTCTAACCTTT
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TATGATGATGATTATTAATATAGTACCAGCCTGTACAGTAGACTCTTTGAAATTTGTTCTCTCTTCACTGAAACTTTTG
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[illegible]

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TGCTGTGATAACATTTCTTTTACTTTTGTAAATGCTTGAATTTTTCATATCTTTTTTCCCCAGAACTTTTTCTTTCCC
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TATCTAGCTAGATCAAGAAATAGCTGATGGTGACTCTGGAAATCCTTGTCTCAAATTATTCATGCTAAGAAATACCACA
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TCTAATATCAGAAATGAAGCAGTATGACAAATAAATATGGTGATTCCATCTGTGCAAAATCACCTGGCATGATCAGTCC
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[illegible]

Fig. 6.305

GAATAAACTCAGTTTGGGAGGGGAGAGAAGTTAATATACAATGCAGAAAAAGTTCAATGAGATCAAAACGTTGGGATCT
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Fig. 6.302

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AAGCTAATTTAAAACTGGTAGAATAGAGGTAACAGAGAATATTGGTATGTCAGCTTCTTTGGTACATTTTCATTTATGA
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Fig. 6.3

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Fig. 6.3347

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CACAGTTCTTTCACAATAGCTTCTTTCTCTCTGTAACCCCTACATAAACTCCAAAAACATTTCTAGTTTTGGAAATCC
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AACATGATCATTCTACTTGTGGGTTTGGACTGACAAGGACATTTCTAGTATATCTTGTGACATCAGGTTAATAAGCT
CTAAGCAAAGCTGAAATGAATGCTACTTCCCAATCAAGTGGAAATGTTAATATTATCAACATGTCTTAAAGGCCAT
GAAGTCCTATGCCTGTATATGTAACAGGAATAGCAAACAGGAACCTTTCTTAAAGGGAAGATTAAACTAGTGTATTTT

Fig. 6.316

[illegible]

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GAGGTTTATAAATGGAACCTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTCT
CTTGCTTTCTCTCTCTCTCTCTCTCTCTCTTTTTTTTTTTTACAAATTGTACTTTAAAGAATAAAGGATCTGAAATC
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TGTATTATTGTATTTGGGGATCAATAAGCAGAGCCAGAGCCTTACACAAGGCTCCTTGTGCCCCCTCCCCGCTCCA
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GCTAAATAAACCAAGCACATAGACCCGAGAATTCTCGTTGATTGATCTTTGGAAATTCGGGTATAAATATGATCAGTCTCAA

Fig. 6.318

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CAAACCTGTTTTCGAAATGAAGGCTTCCCATACATTGAAGTTAAAGTGGTCTTGAGAAAAGAACTTCTTAATAGGTAA
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CTTGAATTGTGGTGCGTCTGCCATTGGTGTGGAAGCAGGGCAGAAAGAGGACAGAGGCATACCAGCAAGTACAGACATC
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GAGGCCATTTTTTTTTGAGTAATCAATATTGTGGAAAAGTGCTAGAGCAGCACTACCTTATATAAATTAATTTTGA
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GGAAAAATAACACTTAAGCAGCTTATTGTATAGCAGAGAGGTGGTTGAATTGATAGACAATTAAAAATACAACGTGTTG
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Fig. 6.32C

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TACCCTAGGGCATCCTTTCTACCAGAGTGACCACCTCGCTTTATGTTTTCTTAGAATAAAACCAATAAATAGTGCAGTT
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Fig. 6.32f

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[illegible]

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Fig. 6.324

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GCTTATT
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CTCAACCAAACTATTGATCAAGAGTGAGCATGGAATAAAGATTATTTCTGAGGTGCGAGCCTTTAAAAATGTATCTCT

Fig. 6.329

GATGCATACACTGACTCAGGAAGCTACTCTGGAAAAACGTGCTCACCAAAAAACAGGGAATAAACTCTAAAAACATTACCCTTGGG
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Fig. 6.330

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CTGTGTTCTCTGCATGTGCTTTTGGAGCCCTTATCACACAGTTGCTCTAGAATTGTTAGCTTTGACCACACTGTGAGTTT
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Fig. 6.133

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Fig. 6.335

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CATCTTAAGTAAATGGTATAACATGAAATCATAGTTTGTGTTCAAGTTATATTTTTTCTGATTTTTTCTAAATTAAT
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CCTTTTACCAACGTAATTTGGGCCAYGTTGCTTCTGTGCTTCAAGTTTCTCATCTGTTAAATAGTTGCTGTGGTGAT
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TATGTGAAGGAATAACATTAGTTTTTATTTTATTTTGGGGGTGAGGGTTAGAGAAGGGTGTCTGCCATCCTGATT

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TCTATCCTTTTGTATAAAGTGACCTAATAATATTGATACTAACCAAGAGGACTATTATAATGTTTTATTACTCTTCTC
CCCCATTAACTACTTTTTTGGTGGTGGTTTTATATGTTTTATTCTTTATTTTTCATCCTTCAAGGAGTTTGCCCTTTGT
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Fig. 6.34

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Fig. 6.344

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Fig. 6.350

[illegible]

Fig. 6.35

[illegible]

Fig. 6.357

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Fig. 6.355

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Position of N ambiguity code

30102 R	150961 Y	290063 R
30205 Y	152214 R	290164 Y
30559 Y	154374 Y	290801 R
30699 K	157074 M	292925 R
34304 R	157272 R	293201 R
34516 K	160863 Y	293611 Y
34782 R	161195 R	295755 R
35697 K	162720 Y	296143 R
35810 Y	163290 R	296739 Y
36817 Y	165441 K	297107 W
40290 K	166462 R	297460 Y
40454 M	168136 Y	297895 R
49148 S	173481 R	298027 Y
55023 Y	173519 R	298152 N
58397 Y	175259 S	298153 N
58622 R	175603 Y	298585 S
58633 S	181225 Y	298605 K
74447 R	197941 M	298799 R
75896 K	198444 Y	299792 M
82244 S	198745 R	300815 Y
88456 W	221134 R	305880 R
88499 R	222532 K	306978 M
90688 S	224195 R	309436 Y
99035 R	224801 Y	309763 Y
102977 R	226923 R	313529 K
104552 Y	227254 Y	313971 R
104862 R	227460 S	317210 S
105225 Y	228326 K	318829 Y
111252 Y	228647 Y	410826 R
111781 Y	228831 R	
112118 M	230175 K	
118914 W	230288 Y	
120628 R	232201 M	
123312 R	232338 M	
123426 S	234332 R	
125304 M	235271 R	
128015 Y	263539 K	
128393 R	270257 R	
129360 Y	270458 Y	
129361 Y	270498 R	
131865 M	271159 Y	
132562 R	274150 Y	
135112 K	274353 M	
138281 Y	275602 Y	
138806 R	277422 M	
147700 Y	278146 R	
147715 R	286615 Y	
148161 Y	289348 S	
148236 Y	289425 R	
148606 K	289868 R	
	289979 Y	

Fig. 6. 358

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<210> 2
 <211> 809
 <212> PRT
 <213> Homo Sapien

<400> 2
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 Trp Arg His Glu Gln His His Gln Tyr Pro Leu Arg Gln Pro Gln Phe
 35 40 45
 Arg Leu Leu His Pro His His His Leu Pro Pro Pro Pro Pro Pro Ser
 50 55 60
 Pro Gln Pro Gln Pro Gln Cys Pro Leu Gln Pro Pro Pro Pro Pro Pro
 65 70 75 80
 Leu Pro Pro Pro Pro Pro Pro Gly Ala Ala Arg Gly Arg Tyr Ala
 85 90 95
 Ser Ser Gly Ala Thr Gly Arg Val Arg His Arg Gly Tyr Ser Asp Thr
 100 105 110
 Glu Arg Tyr Leu Tyr Cys Arg Ala Met Asp Arg Thr Ser Tyr Ala Val
 115 120 125
 Glu Thr Gly His Arg Pro Gly Leu Lys Lys Ser Arg Met Ser Trp Pro
 130 135 140
 Ser Ser Phe Gln Gly Leu Arg Arg Phe Asp Val Asp Asn Gly Thr Ser
 145 150 155 160
 Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
 165 170 175
 Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
 180 185 190
 Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
 195 200 205
 Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
 210 215 220
 Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
 225 230 235 240
 Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
 245 250 255
 Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala
 260 265 270
 Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
 275 280 285
 Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
 290 295 300
 Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
 305 310 315 320
 Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
 325 330 335
 Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
 340 345 350
 Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
 355 360 365
 Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
 370 375 380
 Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
 385 390 395 400
 Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
 405 410 415
 Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
 420 425 430
 Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
 435 440 445
 Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
 450 455 460

Fig. 7.1

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Ile	His	Ala	Ala	Asp	Val	Val	Gln	Ser	Thr	His	Val	Leu	Leu	Ser	Thr
465					470					475					480
Pro	Ala	Leu	Glu	Ala	Val	Phe	Thr	Asp	Leu	Glu	Ile	Leu	Ala	Ala	Ile
				485					490						495
Phe	Ala	Ser	Ala	Ile	His	Asp	Val	Asp	His	Pro	Gly	Val	Ser	Asn	Gln
			500					505					510		
Phe	Leu	Ile	Asn	Thr	Asn	Ser	Glu	Leu	Ala	Leu	Met	Tyr	Asn	Asp	Ser
		515					520					525			
Ser	Val	Leu	Glu	Asn	His	His	Leu	Ala	Val	Gly	Phe	Lys	Leu	Leu	Gln
	530					535					540				
Glu	Glu	Asn	Cys	Asp	Ile	Phe	Gln	Asn	Leu	Thr	Lys	Lys	Gln	Arg	Gln
545					550					555					560
Ser	Leu	Arg	Lys	Met	Val	Ile	Asp	Ile	Val	Leu	Ala	Thr	Asp	Met	Ser
				565					570					575	
Lys	His	Met	Asn	Leu	Leu	Ala	Asp	Leu	Lys	Thr	Met	Val	Glu	Thr	Lys
			580					585					590		
Lys	Val	Thr	Ser	Ser	Gly	Val	Leu	Leu	Asp	Asn	Tyr	Ser	Asp	Arg	
	595						600				605				
Ile	Gln	Val	Leu	Gln	Asn	Met	Val	His	Cys	Ala	Asp	Leu	Ser	Asn	Pro
	610					615					620				
Thr	Lys	Pro	Leu	Gln	Leu	Tyr	Arg	Gln	Trp	Thr	Asp	Arg	Ile	Met	Glu
625					630					635					640
Glu	Phe	Phe	Arg	Gln	Gly	Asp	Arg	Glu	Arg	Glu	Arg	Gly	Met	Glu	Ile
				645					650					655	
Ser	Pro	Met	Cys	Asp	Lys	His	Asn	Ala	Ser	Val	Glu	Lys	Ser	Gln	Val
			660					665					670		
Gly	Phe	Ile	Asp	Tyr	Ile	Val	His	Pro	Leu	Trp	Glu	Thr	Trp	Ala	Asp
	675						680					685			
Leu	Val	His	Pro	Asp	Ala	Gln	Asp	Ile	Leu	Asp	Thr	Leu	Glu	Asp	Asn
	690					695					700				
Arg	Glu	Trp	Tyr	Gln	Ser	Thr	Ile	Pro	Gln	Ser	Pro	Ser	Pro	Ala	Pro
705					710					715					720
Asp	Asp	Pro	Glu	Glu	Gly	Arg	Gln	Gly	Gln	Thr	Glu	Lys	Phe	Gln	Phe
				725					730					735	
Glu	Leu	Thr	Leu	Glu	Glu	Asp	Gly	Glu	Ser	Asp	Thr	Glu	Lys	Asp	Ser
			740					745					750		
Gly	Ser	Gln	Val	Glu	Glu	Asp	Thr	Ser	Cys	Ser	Asp	Ser	Lys	Thr	Leu
	755						760					765			
Cys	Thr	Gln	Asp	Ser	Glu	Ser	Thr	Glu	Ile	Pro	Leu	Asp	Glu	Gln	Val
	770					775					780				
Glu	Glu	Glu	Ala	Val	Gly	Glu	Glu	Glu	Glu	Ser	Gln	Pro	Glu	Ala	Cys
785					790					795					800
Val	Ile	Asp	Asp	Arg	Ser	Pro	Asp	Thr							
					805										

<210> 3
 <211> 150
 <212> PRT
 <213> Homo Sapien

<400> 3
 Met Asp Arg Thr Ser Tyr Ala Val Glu Thr Gly His Arg Pro Gly Leu
 1 5 10 15
 Lys Lys Ser Arg Met Ser Trp Pro Ser Ser Phe Gln Gly Leu Arg Arg
 20 25 30
 Phe Asp Val Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro
 35 40 45
 Met Thr Ser Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val His
 50 55 60
 Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp
 65 70 75 80
 Leu Ser Pro Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile
 85 90 95

Fig. 7.2

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His Gly Asp Asp Leu Ile Val Thr	Pro Phe Ala Gln Val Leu Ala Ser
100	105 110
Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp	
115	120 125
Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn	
130	135 140
Lys Ala Thr Ile Thr Val	
145	150

<210> 4
 <211> 745
 <212> PRT
 <213> Homo Sapien

<400> 4

Met Ala Gln Gln Thr Ser Pro Asp Thr Leu Thr Val Pro Glu Val Asp	
1	5 10 15
Asn Pro His Cys Pro Asn Pro Trp Leu Asn Glu Asp Leu Val Lys Ser	
20	25 30
Leu Arg Glu Asn Leu Leu Gln His Glu Lys Ser Lys Thr Ala Arg Lys	
35	40 45
Ser Val Ser Pro Lys Leu Ser Pro Val Ile Ser Pro Arg Asn Ser Pro	
50	55 60
Arg Leu Leu Arg Arg Met Leu Leu Ser Ser Asn Ile Pro Lys Gln Arg	
65	70 75 80
Arg Phe Thr Val Ala His Thr Cys Phe Asp Val Asp Asn Gly Thr Ser	
85	90 95
Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu	
100	105 110
Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu	
115	120 125
Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg	
130	135 140
Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr	
145	150 155 160
Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe	
165	170 175
Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro	
180	185 190
Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala	
195	200 205
Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu	
210	215 220
Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala	
225	230 235 240
Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser	
245	250 255
Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr	
260	265 270
Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys	
275	280 285
Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys	
290	295 300
Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe	
305	310 315 320
Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp	
325	330 335
Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly	
340	345 350
Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp	
355	360 365
Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu	
370	375 380

Fig. 7.3

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Met	Thr	Leu	Glu	Asp	His	Tyr	His	Ala	Asp	Val	Ala	Tyr	His	Asn	Asn
385					390					395					400
Ile	His	Ala	Ala	Asp	Val	Val	Gln	Ser	Thr	His	Val	Leu	Leu	Ser	Thr
				405					410					415	
Pro	Ala	Leu	Glu	Ala	Val	Phe	Thr	Asp	Leu	Glu	Ile	Leu	Ala	Ala	Ile
			420					425					430		
Phe	Ala	Ser	Ala	Ile	His	Asp	Val	Asp	His	Pro	Gly	Val	Ser	Asn	Gln
		435				440					445				
Phe	Leu	Ile	Asn	Thr	Asn	Ser	Glu	Leu	Ala	Leu	Met	Tyr	Asn	Asp	Ser
450					455						460				
Ser	Val	Leu	Glu	Asn	His	His	Leu	Ala	Val	Gly	Phe	Lys	Leu	Leu	Gln
465				470						475					480
Glu	Glu	Asn	Cys	Asp	Ile	Phe	Gln	Asn	Leu	Thr	Lys	Lys	Gln	Arg	Gln
			485					490						495	
Ser	Leu	Arg	Lys	Met	Val	Ile	Asp	Ile	Val	Leu	Ala	Thr	Asp	Met	Ser
		500					505					510			
Lys	His	Met	Asn	Leu	Leu	Ala	Asp	Leu	Lys	Thr	Met	Val	Glu	Thr	Lys
		515				520					525				
Lys	Val	Thr	Ser	Ser	Gly	Val	Leu	Leu	Leu	Asp	Asn	Tyr	Ser	Asp	Arg
530					535						540				
Ile	Gln	Val	Leu	Gln	Asn	Met	Val	His	Cys	Ala	Asp	Leu	Ser	Asn	Pro
545				550						555					560
Thr	Lys	Pro	Leu	Gln	Leu	Tyr	Arg	Gln	Trp	Thr	Asp	Arg	Ile	Met	Glu
			565					570						575	
Glu	Phe	Phe	Arg	Gln	Gly	Asp	Arg	Glu	Arg	Glu	Arg	Gly	Met	Glu	Ile
		580					585					590			
Ser	Pro	Met	Cys	Asp	Lys	His	Asn	Ala	Ser	Val	Glu	Lys	Ser	Gln	Val
		595				600					605				
Gly	Phe	Ile	Asp	Tyr	Ile	Val	His	Pro	Leu	Trp	Glu	Thr	Trp	Ala	Asp
610					615						620				
Leu	Val	His	Pro	Asp	Ala	Gln	Asp	Ile	Leu	Asp	Thr	Leu	Glu	Asp	Asn
625				630					635						640
Arg	Glu	Trp	Tyr	Gln	Ser	Thr	Ile	Pro	Gln	Ser	Pro	Ser	Pro	Ala	Pro
			645					650						655	
Asp	Asp	Pro	Glu	Gly	Arg	Gln	Gly	Gln	Thr	Glu	Lys	Phe	Gln	Phe	
		660					665					670			
Glu	Leu	Thr	Leu	Glu	Glu	Asp	Gly	Glu	Ser	Asp	Thr	Glu	Lys	Asp	Ser
		675				680					685				
Gly	Ser	Gln	Val	Glu	Glu	Asp	Thr	Ser	Cys	Ser	Asp	Ser	Lys	Thr	Leu
690				695							700				
Cys	Thr	Gln	Asp	Ser	Glu	Ser	Thr	Glu	Ile	Pro	Leu	Asp	Glu	Gln	Val
705				710						715					720
Glu	Glu	Glu	Ala	Val	Gly	Glu	Glu	Glu	Glu	Ser	Gln	Pro	Glu	Ala	Cys
			725					730						735	
Val	Ile	Asp	Asp	Arg	Ser	Pro	Asp	Thr							
			740					745							

<210> 5
 <211> 215
 <212> PRT
 <213> Homo Sapien

<400> 5

Met	Ala	Gln	Gln	Thr	Ser	Pro	Asp	Thr	Leu	Thr	Val	Pro	Glu	Val	Asp
1				5					10					15	
Asn	Pro	His	Cys	Pro	Asn	Pro	Trp	Leu	Asn	Glu	Asp	Leu	Val	Lys	Ser
			20					25					30		
Leu	Arg	Glu	Asn	Leu	Leu	Gln	His	Glu	Lys	Ser	Lys	Thr	Ala	Arg	Lys
		35				40						45			
Ser	Val	Ser	Pro	Lys	Leu	Ser	Pro	Val	Ile	Ser	Pro	Arg	Asn	Ser	Pro
	50				55					60					
Arg	Leu	Leu	Arg	Arg	Met	Leu	Leu	Ser	Ser	Asn	Ile	Pro	Lys	Gln	Arg
65					70					75					80

Fig. 7.4

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Arg Phe Thr Val Ala His Thr Cys Phe Asp Val Asp Asn Gly Thr Ser
 85 90 95
 Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
 100 105 110
 Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
 115 120 125
 Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
 130 135 140
 Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
 145 150 155 160
 Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
 165 170 175
 Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
 180 185 190
 Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Gly Leu Tyr
 195 200 205
 Asn Gly Ile Ile Ala Phe Leu
 210 215

<210> 6
 <211> 673
 <212> PRT
 <213> Homo Sapien

<400> 6
 Met Met His Val Asn Asn Phe Pro Phe Arg Arg His Ser Trp Ile Cys
 1 5 10 15
 Phe Asp Val Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro
 20 25 30
 Met Thr Ser Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val His
 35 40 45
 Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp
 50 55 60
 Leu Ser Pro Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile
 65 70 75 80
 His Gly Asp Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala Ser
 85 90 95
 Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp
 100 105 110
 Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn
 115 120 125
 Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr
 130 135 140
 Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr
 145 150 155 160
 Arg His Ser Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu
 165 170 175

 Asn Arg Glu Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln
 180 185 190
 Val Ser Glu Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val
 195 200 205
 Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Arg Pro
 210 215 220
 Met Ser Gln Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu
 225 230 235 240
 Thr Asn Ser Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp
 245 250 255
 Val Leu Ala Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His Val
 260 265 270
 Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile Met
 275 280 285
 His Thr Ile Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro
 290 295 300

Fig. 7.5

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Val	Asp	Thr	Leu	Ile	Thr	Tyr	Leu	Met	Thr	Leu	Glu	Asp	His	Tyr	His
305					310					315					320
Ala	Asp	Val	Ala	Tyr	His	Asn	Asn	Ile	His	Ala	Ala	Asp	Val	Val	Gln
			325						330					335	
Ser	Thr	His	Val	Leu	Leu	Ser	Thr	Pro	Ala	Leu	Glu	Ala	Val	Phe	Thr
			340					345					350		
Asp	Leu	Glu	Ile	Leu	Ala	Ala	Ile	Phe	Ala	Ser	Ala	Ile	His	Asp	Val
		355					360					365			
Asp	His	Pro	Gly	Val	Ser	Asn	Gln	Phe	Leu	Ile	Asn	Thr	Asn	Ser	Glu
	370					375					380				
Leu	Ala	Leu	Met	Tyr	Asn	Asp	Ser	Ser	Val	Leu	Glu	Asn	His	His	Leu
385					390					395					400
Ala	Val	Gly	Phe	Lys	Leu	Leu	Gln	Glu	Glu	Asn	Cys	Asp	Ile	Phe	Gln
				405					410					415	
Asn	Leu	Thr	Lys	Lys	Gln	Arg	Gln	Ser	Leu	Arg	Lys	Met	Val	Ile	Asp
			420				425						430		
Ile	Val	Leu	Ala	Thr	Asp	Met	Ser	Lys	His	Met	Asn	Leu	Leu	Ala	Asp
		435				440						445			
Leu	Lys	Thr	Met	Val	Glu	Thr	Lys	Lys	Val	Thr	Ser	Ser	Gly	Val	Leu
	450					455					460				
Leu	Leu	Asp	Asn	Tyr	Ser	Asp	Arg	Ile	Gln	Val	Leu	Gln	Asn	Met	Val
465					470					475					480
His	Cys	Ala	Asp	Leu	Ser	Asn	Pro	Thr	Lys	Pro	Leu	Gln	Leu	Tyr	Arg
			485						490					495	
Gln	Trp	Thr	Asp	Arg	Ile	Met	Glu	Glu	Phe	Phe	Arg	Gln	Gly	Asp	Arg
			500					505					510		
Glu	Arg	Glu	Arg	Gly	Met	Glu	Ile	Ser	Pro	Met	Cys	Asp	Lys	His	Asn
		515				520						525			
Ala	Ser	Val	Glu	Lys	Ser	Gln	Val	Gly	Phe	Ile	Asp	Tyr	Ile	Val	His
	530					535					540				
Pro	Leu	Trp	Glu	Thr	Trp	Ala	Asp	Leu	Val	His	Pro	Asp	Ala	Gln	Asp
545					550					555					560
Ile	Leu	Asp	Thr	Leu	Glu	Asp	Asn	Arg	Glu	Trp	Tyr	Gln	Ser	Thr	Ile
			565						570					575	
Pro	Gln	Ser	Pro	Ser	Pro	Ala	Pro	Asp	Asp	Pro	Glu	Glu	Gly	Arg	Gln
			580					585					590		
Gly	Gln	Thr	Glu	Lys	Phe	Gln	Phe	Glu	Leu	Thr	Leu	Glu	Glu	Asp	Gly
		595				600						605			
Glu	Ser	Asp	Thr	Glu	Lys	Asp	Ser	Gly	Ser	Gln	Val	Glu	Glu	Asp	Thr
	610					615					620				
Ser	Cys	Ser	Asp	Ser	Lys	Thr	Leu	Cys	Thr	Gln	Asp	Ser	Glu	Ser	Thr
625					630					635					640
Glu	Ile	Pro	Leu	Asp	Glu	Gln	Val	Glu	Glu	Glu	Ala	Val	Gly	Glu	Glu
			645						650					655	
Glu	Glu	Ser	Gln	Pro	Glu	Ala	Cys	Val	Ile	Asp	Asp	Arg	Ser	Pro	Asp
			660					665					670		

Thr

<210> 7
 <211> 15
 <212> PRT
 <213> Homo Sapien

<400> 7
 Met Met His Val Asn Asn Phe Pro Phe Arg Arg His Ser Trp Ile
 1 5 10 15

<210> 8
 <211> 687
 <212> PRT
 <213> Homo Sapien

<400> 8

Fig. 7.6

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Met	Ala	Phe	Val	Trp	Asp	Pro	Leu	Gly	Ala	Thr	Val	Pro	Gly	Pro	Ser
1				5					10					15	
Thr	Arg	Ala	Lys	Ser	Arg	Leu	Arg	Phe	Ser	Lys	Ser	Tyr	Ser	Phe	Asp
			20					25					30		
Val	Asp	Asn	Gly	Thr	Ser	Ala	Gly	Arg	Ser	Pro	Leu	Asp	Pro	Met	Thr
		35					40					45			
Ser	Pro	Gly	Ser	Gly	Leu	Ile	Leu	Gln	Ala	Asn	Phe	Val	His	Ser	Gln
		50				55				60					
Arg	Arg	Glu	Ser	Phe	Leu	Tyr	Arg	Ser	Asp	Ser	Asp	Tyr	Asp	Leu	Ser
65					70					75				80	
Pro	Lys	Ser	Met	Ser	Arg	Asn	Ser	Ser	Ile	Ala	Ser	Asp	Ile	His	Gly
				85					90					95	
Asp	Asp	Leu	Ile	Val	Thr	Pro	Phe	Ala	Gln	Val	Leu	Ala	Ser	Leu	Arg
			100					105					110		
Thr	Val	Arg	Asn	Asn	Phe	Ala	Ala	Leu	Thr	Asn	Leu	Gln	Asp	Arg	Ala
			115					120				125			
Pro	Ser	Lys	Arg	Ser	Pro	Met	Cys	Asn	Gln	Pro	Ser	Ile	Asn	Lys	Ala
						135					140				
Thr	Ile	Thr	Glu	Glu	Ala	Tyr	Gln	Lys	Leu	Ala	Ser	Glu	Thr	Leu	Glu
145					150					155				160	
Glu	Leu	Asp	Trp	Cys	Leu	Asp	Gln	Leu	Glu	Thr	Leu	Gln	Thr	Arg	His
				165					170					175	
Ser	Val	Ser	Glu	Met	Ala	Ser	Asn	Lys	Phe	Lys	Arg	Met	Leu	Asn	Arg
				180				185					190		
Glu	Leu	Thr	His	Leu	Ser	Glu	Met	Ser	Arg	Ser	Gly	Asn	Gln	Val	Ser
			195					200				205			
Glu	Phe	Ile	Ser	Asn	Thr	Phe	Leu	Asp	Lys	Gln	His	Glu	Val	Glu	Ile
					215						220				
Pro	Ser	Pro	Thr	Gln	Lys	Glu	Lys	Glu	Lys	Lys	Lys	Arg	Pro	Met	Ser
225					230					235				240	
Gln	Ile	Ser	Gly	Val	Lys	Lys	Leu	Met	His	Ser	Ser	Ser	Leu	Thr	Asn
				245					250					255	
Ser	Ser	Ile	Pro	Arg	Phe	Gly	Val	Lys	Thr	Glu	Gln	Glu	Asp	Val	Leu
			260					265					270		
Ala	Lys	Glu	Leu	Glu	Asp	Val	Asn	Lys	Trp	Gly	Leu	His	Val	Phe	Arg
			275				280					285			
Ile	Ala	Glu	Leu	Ser	Gly	Asn	Arg	Pro	Leu	Thr	Val	Ile	Met	His	Thr
			290			295					300				
Ile	Phe	Gln	Glu	Arg	Asp	Leu	Leu	Lys	Thr	Phe	Lys	Ile	Pro	Val	Asp
305					310					315				320	
Thr	Leu	Ile	Thr	Tyr	Leu	Met	Thr	Leu	Glu	Asp	His	Tyr	His	Ala	Asp
				325					330					335	
Val	Ala	Tyr	His	Asn	Asn	Ile	His	Ala	Ala	Asp	Val	Val	Gln	Ser	Thr
			340					345					350		
His	Val	Leu	Leu	Ser	Thr	Pro	Ala	Leu	Glu	Ala	Val	Phe	Thr	Asp	Leu
			355				360					365			
Glu	Ile	Leu	Ala	Ala	Ile	Phe	Ala	Ser	Ala	Ile	His	Asp	Val	Asp	His
			370			375					380				
Pro	Gly	Val	Ser	Asn	Gln	Phe	Leu	Ile	Asn	Thr	Asn	Ser	Glu	Leu	Ala
385					390					395				400	
Leu	Met	Tyr	Asn	Asp	Ser	Ser	Val	Leu	Glu	Asn	His	His	Leu	Ala	Val
				405					410					415	
Gly	Phe	Lys	Leu	Leu	Gln	Glu	Glu	Asn	Cys	Asp	Ile	Phe	Gln	Asn	Leu
			420					425					430		
Thr	Lys	Lys	Gln	Arg	Gln	Ser	Leu	Arg	Lys	Met	Val	Ile	Asp	Ile	Val
			435				440					445			
Leu	Ala	Thr	Asp	Met	Ser	Lys	His	Met	Asn	Leu	Leu	Ala	Asp	Leu	Lys
			450			455				460					
Thr	Met	Val	Glu	Thr	Lys	Lys	Val	Thr	Ser	Ser	Gly	Val	Leu	Leu	Leu
465					470					475				480	
Asp	Asn	Tyr	Ser	Asp	Arg	Ile	Gln	Val	Leu	Gln	Asn	Met	Val	His	Cys
				485					490					495	
Ala	Asp	Leu	Ser	Asn	Pro	Thr	Lys	Pro	Leu	Gln	Leu	Tyr	Arg	Gln	Trp
			500					505					510		
Thr	Asp	Arg	Ile	Met	Glu	Glu	Phe	Phe	Arg	Gln	Gly	Asp	Arg	Glu	Arg
			515				520					525			

Fig. 7.7

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Glu Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn Ala Ser
 530 535 540
 Val Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His Pro Leu
 545 550 555 560
 Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp Ile Leu
 565 570 575
 Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln
 580 585 590
 Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln
 595 600 605
 Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser
 610 615 620
 Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr Ser Cys
 625 630 635 640
 Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile
 645 650 655
 Pro Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu Glu Glu
 660 665 670
 Ser Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp Thr
 675 680 685

<210> 9
 <211> 585
 <212> PRT
 <213> Homo Sapien

<400> 9
 Met Lys Glu Gln Pro Ser Cys Ala Gly Thr Gly His Pro Ser Met Ala
 1 5 10 15
 Gly Tyr Gly Arg Met Ala Pro Phe Glu Leu Ala Ser Gly Pro Val Lys
 20 25 30
 Arg Leu Arg Thr Glu Ser Pro Phe Pro Cys Leu Phe Ala Glu Glu Ala
 35 40 45
 Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
 50 55 60
 Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
 65 70 75 80
 Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
 85 90 95
 Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
 100 105 110
 Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
 115 120 125
 Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
 130 135 140
 Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
 145 150 155 160
 Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
 165 170 175
 Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
 180 185 190
 Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
 195 200 205
 Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
 210 215 220
 Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
 225 230 235 240
 Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
 245 250 255
 Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
 260 265 270
 Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
 275 280 285
 Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
 290 295 300

Fig. 7.8

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Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
305          310          315          320
Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
          325          330          335
Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
          340          345          350
Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
          355          360          365
Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
          370          375          380
Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
385          390          395          400
Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
          405          410          415
Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
          420          425          430
Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
          435          440          445
Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
          450          455          460
Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
465          470          475          480
Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
          485          490          495
Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
          500          505          510
Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
          515          520          525
Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu
          530          535          540
Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
545          550          555          560
Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys
          565          570          575
Val Ile Asp Asp Arg Ser Pro Asp Thr
          580          585

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<210> 10
 <211> 507
 <212> PRT
 <213> Homo Sapien

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<400> 10
Met Ala Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His
1          5          10          15
Leu Ser Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser
          20          25          30
Asn Thr Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr
          35          40          45
Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly
          50          55          60
Val Lys Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro
65          70          75          80
Arg Phe Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu
          85          90          95
Glu Asp Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu
          100          105          110
Ser Gly Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu
          115          120          125
Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr
          130          135          140
Tyr Leu Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His
145          150          155          160
Asn Asn Ile His Ala Asp Val Val Gln Ser Thr His Val Leu Leu
          165          170          175

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Fig. 7.9

Ser	Thr	Pro	Ala	Leu	Glu	Ala	Val	Phe	Thr	Asp	Leu	Glu	Ile	Leu	Ala
			180					185					190		
Ala	Ile	Phe	Ala	Ser	Ala	Ile	His	Asp	Val	Asp	His	Pro	Gly	Val	Ser
		195					200					205			
Asn	Gln	Phe	Leu	Ile	Asn	Thr	Asn	Ser	Glu	Leu	Ala	Leu	Met	Tyr	Asn
	210					215					220				
Asp	Ser	Ser	Val	Leu	Glu	Asn	His	His	Leu	Ala	Val	Gly	Phe	Lys	Leu
225					230					235					240
Leu	Gln	Glu	Glu	Asn	Cys	Asp	Ile	Phe	Gln	Asn	Leu	Thr	Lys	Lys	Gln
				245					250					255	
Arg	Gln	Ser	Leu	Arg	Lys	Met	Val	Ile	Asp	Ile	Val	Leu	Ala	Thr	Asp
			260					265					270		
Met	Ser	Lys	His	Met	Asn	Leu	Leu	Ala	Asp	Leu	Lys	Thr	Met	Val	Glu
		275					280					285			
Thr	Lys	Lys	Val	Thr	Ser	Ser	Gly	Val	Leu	Leu	Leu	Asp	Asn	Tyr	Ser
	290					295					300				
Asp	Arg	Ile	Gln	Val	Leu	Gln	Asn	Met	Val	His	Cys	Ala	Asp	Leu	Ser
305					310					315					320
Asn	Pro	Thr	Lys	Pro	Leu	Gln	Leu	Tyr	Arg	Gln	Trp	Thr	Asp	Arg	Ile
				325					330					335	
Met	Glu	Glu	Phe	Phe	Arg	Gln	Gly	Asp	Arg	Glu	Arg	Glu	Arg	Gly	Met
			340					345					350		
Glu	Ile	Ser	Pro	Met	Cys	Asp	Lys	His	Asn	Ala	Ser	Val	Glu	Lys	Ser
		355					360					365			
Gln	Val	Gly	Phe	Ile	Asp	Tyr	Ile	Val	His	Pro	Leu	Trp	Glu	Thr	Trp
	370					375					380				
Ala	Asp	Leu	Val	His	Pro	Asp	Ala	Gln	Asp	Ile	Leu	Asp	Thr	Leu	Glu
385					390					395					400
Asp	Asn	Arg	Glu	Trp	Tyr	Gln	Ser	Thr	Ile	Pro	Gln	Ser	Pro	Ser	Pro
				405					410					415	
Ala	Pro	Asp	Asp	Pro	Glu	Glu	Gly	Arg	Gln	Gly	Gln	Thr	Glu	Lys	Phe
			420					425					430		
Gln	Phe	Glu	Leu	Thr	Leu	Glu	Glu	Asp	Gly	Glu	Ser	Asp	Thr	Glu	Lys
		435					440					445			
Asp	Ser	Gly	Ser	Gln	Val	Glu	Glu	Asp	Thr	Ser	Cys	Ser	Asp	Ser	Lys
	450					455					460				
Thr	Leu	Cys	Thr	Gln	Asp	Ser	Glu	Ser	Thr	Glu	Ile	Pro	Leu	Asp	Glu
465					470					475					480
Gln	Val	Glu	Glu	Glu	Ala	Val	Gly	Glu	Glu	Glu	Glu	Ser	Gln	Pro	Glu
				485					490					495	
Ala	Cys	Val	Ile	Asp	Asp	Arg	Ser	Pro	Asp	Thr					
			500					505							

Fig. 7.10

Exon start	Exon end	Exons	Isoform	142207	444645	444775	641649	736254	861791	1044051	1273404	1354347	1414511	1436943	1445217
mRNA/cDNA variants				142328	444775	641878	737226	862202	1044190	1273709	1355128	1414702	1445290		
				4D7-1	4D7-2	4D7-3	4D4	4D5	4D3	4D6	4D8	LF1	LF2	LF3	
UO2882															
L20969			4D4				*					*	*	*	*
AF012073			4D5					*				*	*	*	*
L20970			4D3						*			*	*	*	*
AF012074			4D2												
U50159			4D3						*			*	*	*	*
U50158			4D2												
U50157			4D1												
AJ250854			4DN3					*				*	*	*	*
NM_006203			4D4				*					*	*	*	*
AJ250852			4DN1									*	*	*	*
AJ250855			4DN2				*					*	*	*	*
BC008390			4DN3					*				*	*	*	*
novel cDNA identified by deCODE															
RT-PCR			4D6							*			*	*	*
CAP-RACE			4D7	*	*	*						*	*	*	*
CAP-RACE			4D8								*		*	*	*

Fig. 8A

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[illegible]

Fig. 8B

